HANDBOOK OF
Food Products Manufacturing

Principles, Bakery, Beverages, Cereals, Cheese, Confectionary, Fats, Fruits, and Functional Foods

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Handbook of Food Products Manufacturing

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WILEY-INTERSCIENCE
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Contents

Principles, Bakery, Beverages, Cereals, Cheese, Confectionary, Fats, Fruits, and Functional Foods

PREFACE ix

CONTRIBUTORS LIST xiii

PART A: FOOD MANUFACTURING: BACKGROUND 1

Section I: Principles and Establishments Classification 3

1. Fundamentals of Food Manufacturing 5
Wai-Kit Nip

2. Fermented Products and Their Manufacture 45
Wai-Kit Nip

3. Food Manufacturing in the United States: Standard Industrial Classification 85
Y.H. Hui

Section II: Flavors: Food Processing, Product Developments, and Recent Advances 117

4. Food Flavorings: Principles of Applications 119
Taiwo O. Omobuwajo

5. Product Development 131
Taiwo O. Omobuwajo

6. Extraction Modes 147
Marisa F. Mendes, Fernando L.P. Pessoa, Sílvio A.B. Vieira De Melo, and Eduardo M. Queiroz

7. Distillation and Drying 157
Fernando L.P. Pessoa, Marisa F. Mendes, Eduardo M. Queiroz, Sílvio A.B. Vieira de Melo, and David Lee Nelson

8. Genetic Engineering 169
Gláucia Maria Pastore and Gabriela Alves Macedo

9. Flavor Compounds Produced by Fungi, Yeasts, and Bacteria 179
Carlos R. Soccol, Adriane B.P. Medeiros, Luciana P.S. Vandenberghe, and Adenise L. Woiciechowski

10. Flavor Production by Solid and Liquid Fermentation 193
Carlos R. Soccol, Adriane B.P. Medeiros, Luciana P.S. Vandenberghe, and Adenise L. Woiciechowski

11. Herbs, Spices, and Essential Oils 205
Alain Darriet

Section III: Food Sanitation and Establishment Inspection 221

12. FDA GMPs, HACCP, and the Food Code 223
Nanna Cross

13. Food Establishment Inspection 245
Y.H. Hui

PART B: FOOD PRODUCTS MANUFACTURING 257

Section IV: Bakery Products 259

14. Manufacturing of Bread and Bakery Products 261
Weibiao Zhou and Nantawan Therdthai

15. Muffins and Bagels 279
Nanna Cross

16. Fundamentals of Cakes: Ingredients and Production 307
Frank D. Conforti

17. Traditional Italian Products from Wheat and Other Starchy Flours 327
M. Ambrogina Pagani, Mara Lucisano, and Manuela Mariotti

18. Flavor Migration in Solid Food Matrices 389
Yi-Chung Fu

Section V: Beverages 417

19. Carbonated Beverages 419
Daniel W. Bena

20. The Beer Brewing Process: Wort Production and Beer Fermentation 443
Ronnie Willaert

21. Manufacture of Whisky 507
Takefumi Yoneya

Section VI: Cereals: Rice and Noodles 521

22. Rice-Based Products 523
C.M. Rosell and C. Collar

23. Asian (Oriental) Noodles and Their Manufacture 539
Wai-Kit Nip

Section VII: Cheeses 565

24. Cheddar and Related Hard Cheeses 567
Stephanie Clark and Shantanu Agarwal

25. Pasteurized Process and Related Cheeses 595
Stephanie Clark and Shantanu Agarwal

26. Cottage Cheese 617
Stephanie Clark and David E. Potter

27. Cheese Varieties Made by Direct Acidification of Hot Milk 635
Ramesh C. Chandan

28. Cream Cheese as an Acidified Protein-Stabilized Emulsion Gel 651
Arjen Bot, Frank A.M. Kleinherenbrink, Michel Mellema, and Christel K. Magnani

Section VIII: Confectionery 673

29. Chocolate and Cocoa 675
Randall Hofberger and Nina Ann Tanabe

30. Confectionery: Inspection and Enforcement 695
Y.H. Hui

Section IX: Fats and Oils 703

31. Margarine and Dairy Spreads: Processing and Technology 705
Pernille Gerstenberg Kirkeby

32. Cream Products **725**
David W. Everett

33. Influence of Processing on Virgin Olive Oil Quality **751**
Maria Roca, Beatriz Gandul-Rojas, and M. Isabel Mínguez-Mosquera

Section X: Fruits and Fruit Juices **771**

34. Apple: Production, Chemistry, and Processing **773**
Nirmal K. Sinha

35. Strawberries and Blueberries: Phytonutrients and Products **793**
Nirmal K. Sinha

36. Major Tropical Fruits and Products: Banana, Mango, and Pineapple **815**
Lillian G. Po

37. Fruit Juices **847**
Terri D. Boylston

38. Dried Banana **867**
Raquel Pinho Ferreira Guiné and Maria João Barroca Dias

39. Fresh-Cut Fruits **879**
Olga Martín-Belloso, Robert Soliva-Fortuny, and Gemma Oms-Oliu

Section XI: Functional Foods **901**

40. Functional Foods and Ingredients **903**
Chung-Ja C. Jackson and Gopinadhan Paliyath

41. Functional Foods: International Considerations **939**
Kelley Fitzpatrick

42. Functional Foods Based on Dairy Ingredients **957**
Ramesh C. Chandan and N.P. Shah

43. Functional Properties of Milk Constituents **971**
Ramesh C. Chandan

44. Functional Foods Based on Meat Products **989**
Francisco Jiménez-Comenero

45. Gluten-Free Cereal Products as a Functional Food **1017**
Elke K. Arendt and Michelle M. Moore

VOLUME 1 INDEX **1037**

VOLUME 1 CONTENTS **1065**

VOLUME 2 CONTENTS **1069**

CUMULATIVE INDEX **1073**

Contents

Health, Meat, Milk, Poultry, Seafood, and Vegetables

PREFACE ix

CONTRIBUTORS xiii

PART B: FOOD PRODUCTS MANUFACTURING (CONTINUED) 1

Section XII: Health Ingredients and Health Products Development for Preventing or Treating Human Diseases 3

46. Bioactive Peptides from Food Proteins 5
Hannu Korhonen and Anne Pihlanto

47. Lipid-Soluble Vitamins: Nutritional and Functional Aspects 39
Antonio Pérez-Gálvez and María Isabel Mínguez-Mosquera

48. Phytochemicals in Mediterranean Diet: The Interaction Between Tomato and Olive Oil Bioactive Compounds 55
Rita Pernice, Paola Vitaglione, Raffaele Sacchi, and Vincenzo Fogliano

49. Functional Microbes: Technology for Health Foods 67
Maria Saarela and Tiina Mattila-Sandholm

50. Enterococci and Dairy Products 85
Giorgio Giraffa

51. Fermented Milk: Health Benefits Beyond Probiotic Effect 99
Todor Vasiljevic and Nagendra P. Shah

52. Application of Functional Foods and Nutraceuticals in Allergic Disorders: Principles and Potential **117**
Venu Gangur

53. Functional Foods, Herbs, and Aging **135**
Carlos Kusano Bucalen Ferrari

54. Functional Foods and Gastrointestinal Disorders **153**
Jude Ryan, Shomik Sibartie, Liam O'Mahony, and Fergus Shanahan

55. Herbs in the Management of Diabetes Mellitus with an Emphasis on Ginseng **175**
Vladimir Vuksan and John L. Sievenpiper

56. Functional Foods and Minerals: Calcium **201**
John J.B. Anderson

Section XIII: Meat and Meat Products **215**

57. Thermal Processing **217**
Isabel Guerrero Legarreta

58. Ham **233**
Fidel Toldrá

59. Sausages **251**
Fidel Toldrá and Milagro Reig

60. Fermented Meat Production **265**
Fidel Toldrá

61. Processed Pork Meat Flavors **281**
Fidel Toldrá and Mónica Flores

62. Sensory Quality of Meat Products **303**
Fidel Toldrá and Mónica Flores

Section XIV: Milk and Milk Products **329**

63. Processing Quality Fluid Milk Products **331**
James V. Chambers and Somjit Surapat

64. Milk Composition, Physical and Processing Characteristics **347**
Ramesh C. Chandan

65. Genetics and Milk Production **379**
Stephanie Clark and Michael J. Costello

66. Flavored Milks **403**
Kayanush J. Aryana

67. Fermented Milks Popular in Europe and North America **411**
A.Y. Tamime and R.K. Robinson

68. Fermented Milk in Asia **431**
Sardar Iftekharudin Ahmed and Jutatip Wangsai

69. Goat Milk, Its Products and Nutrition **449**
Young W. Park and George F.W. Haenlein

70. Bioactive Peptides in Dairy Products **489**
Marco Gobbetti, Fabio Minervini, and Carlo Giuseppe Rizzello

71. Science and Technology of Sour Cream **519**
Wilfried Rieder

72. Dairy Protein Hydrolysates **537**
Ifendu A. Nnanna and Chao Wu

73. The Manufacture and Applications of Casein-Derived Ingredients **557**
John E. O'Connell and Cal Flynn

74. Ice Cream and Frozen Desserts **593**
Arun Kilara and Ramesh C. Chandan

75. Frozen Novelties **635**
Jonathan Hopkinson

76. Yogurt **647**
Lisa Pannell and Tonya C. Schoenfuss

77. Infant Formulas **677**
G. Boehm, C. Beermann, G. Georgi, and B. Stahl

78. Biochemical Processes in the Production of Flavor in Milk and Milk Products **715**
Tanoj K. Singh, Keith R. Cadwallader, and MaryAnne Drake

Section XV: Poultry Products **749**

79. Poultry Marination **751**
Edith Ponce-Alquicira

80. Poultry Sausages **773**
Alfonso Totosaus and M. Lourdes Pérez-Chabela

81. Flavor of Fresh and Frozen Poultry **781**
Edith Ponce-Alquicira

82. Edible Packaging for Poultry and Poultry Products **795**
Baciliza Quintero-Salazar and Edith Ponce-Alquicira

Section XVI: Seafood **815**

83. Frozen Seafood Products Description **817**
Nanna Cross

84. Processing Frozen Seafoods **855**
Shann-Tzong Jiang and Tung-Ching Lee

85. Shellfish Freezing **893**
Athapol Noomhorm, Mukku Shrinivas Rao, and Punchira Vongsawasdi

86. Processing Formulated Fish and Fish Products **915**
Jamilah Bakar

87. Flavorants from Seafood Byproducts **931**
Rosa Jonsdottir, Gudrun Olafsdottir, Sigurður Hauksson, and Jon Magnus Einarsson

88. Seafood Processing: Basic Sanitation Practices **947**
Nanna Cross

89. Sustainable Intensive Aquaculture **969**
C.G. Carter

Section XVII: Vegetable Products **983**

90. Frozen Vegetables and Product Descriptions **985**
Nanna Cross

91. Frozen Avocados **997**
Rosa María Galicia-Cabrera

92. Frozen French Fried Potatoes and Quality Assurance **1013**
Nanna Cross

93. Paprika Production: Current Processing Techniques and Emerging Technologies **1031**
María Isabel Mínguez-Mosquera, Manuel Jarén-Galán, Dámaso Hornero-Méndez, and Antonio Pérez-Gálvez

94. Pickles Manufacturing in the United States: Quality Assurance and Establishment Inspection **1045**
Nanna Cross

95. Soymilk and Tofu Manufacturing **1063**
Sam K.C. Chang

96. Tomato Processing **1091**
Rosa María Galicia-Cabrera

97. Dried Tomato **1109**
Raquel Pinho Ferreira Guiné and Maria João Barroca Dias

VOLUME 2 INDEX **1123**

VOLUME 1 CONTENTS **1155**

VOLUME 2 CONTENTS **1159**

CUMULATIVE INDEX **1163**

Preface

It is obvious that professionals from the government, industry, and academia need reference books on the manufacturing of food products. Publishers serve this market by publishing three categories of books:

1. Books on the principles of food processing with or without a discussion of manufacturing selected food commodities. The discussion of the manufacturing of specific food products, if included, is usually limited to a representative few.
2. Books on the processing of one category of food products, for example, bakery, seafood, poultry. There are hundreds of such books in the market.
3. Multivolume reference books such as handbooks and encyclopedia covering all areas of food science, technology, and engineering, including the manufacturing of selected food products. Obviously, the number of food products included is also limited.

The cost and the relative need of multivolume reference books in food science, technology, and engineering make the purchase of books under the third group unrealistic and prohibitive for many libraries and personal bookshelves. The *Handbook of Food Products Manufacturing* is designed to fill the gap in the above book categories in terms of relative cost and coverage. It is presented in two volumes and has the following profiles:

- Covers the manufacturing of selected food products and the process of development of selected food products from specific food ingredients.
- Supplements many food processing texts already in the market.
- Serves as a general reference treatise of a reasonable size and cost that will provide information on manufacturing of some food products and topics of related interest.

One can appreciate the difficulty of this task from the simple observation that books with the same coverage are not common. The editors of this proposal, with the help of professionals from academia, government, and industry, hope to achieve these objectives. However, it is important to emphasize the following premises:

1. The coverage is not entirely comprehensive if one considers the wide spectrum of food products in the market. Additionally, scientific and technological advancement in food processing will always outpace publishing.
2. Several factors determine the selection of topics coverage:
   i. Some products are popular for many consumers.
   ii. Some products are seldom covered in other books on similar topics.
   iii. Expertise of members of the editorial team.
   iv. Availability of chapter contributors in terms of time, background, expertise, etc.
3. Since this is a general reference text, the depth of coverage is limited to competencies associated with general users rather than specialists.

Based on the above premises, these two volume texts cover the following chapters:

- Fifty-four chapters on the direct manufacturing of specific food products. These chapters cover: bakery, beverages, cheese, confectionery, fats and oils, fruits, meats, milk and dairy products, poultry, seafood, and vegetables.
- Thirty-two chapters on the use of specific food ingredients to develop food products new or otherwise. These chapters cover products development from many known food ingredients. The products to be developed cover traditional foods (food flavors, bakery, fruit juices, milk and dairy products, and poultry products) and new foods (fresh-cut fruits, functional foods for consumers, and medical foods for treating and preventing diseases).
- Five chapters on the recent development of food flavors in view of the role of food flavors in all aspects of food manufacturing. These chapters cover extraction, distillation, biotechnology, and other topics.
- Five chapters on legal requirements in the United States for food classification and manufacturing of food products such as good manufacturing practice (GMP), hazard analysis and critical control points (HACCP), and establishment inspection.

Although this book is relevant to many professions, it will probably be most useful to:

1. Undergraduate and graduate students for research for classroom assignments or preliminary research investigation for potential topics.
2. Professionals from industry, government, and academia interested in topics peripheral to their specialties.
3. Small start-up food manufacturing companies looking for potential products for development.

From the above list of potential users it is obvious that this book is uniquely designed for private or public libraries, small or large. This work is the result of the combined efforts of nearly 200 professionals from industry, government, and academia worldwide. They represent more than 35 countries with diverse expertise and background in the science and

technology of food manufacturing and food products development. An international editorial team of 13 members from five countries led these experts. Each contributor or editor was responsible for researching and reviewing subjects of immense depth, breadth and complexity. Care and attention were paramount to ensure technical accuracy for each topic. In sum, this volume is unique. It is our sincere hope and expectation that it will serve as an essential reference on the manufacturing of food products, for professionals in government, industry, and academia.

The editorial team wishes to thank all the contributors for sharing their expertise throughout our journey. We also thank the reviewers for giving their valuable comments on how to improve the contents of each chapter. All these professionals made this book possible. We trust that you will benefit from the fruits of their labor.

We know firsthand the challenges in developing a book of this scope. What follows are the difficulties in producing the book. We thank the editorial and production teams at John Wiley, Inc. and Techset Composition Ltd., Salisbury, UK, for their time, effort, advice, and expertise. You are the best judge of the quality of this work.

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Contents

Principles, Bakery, Beverages, Cereals, Cheese, Confectionary, Fats, Fruits, and Functional Foods

PREFACE ix

CONTRIBUTORS LIST xiii

PART A: FOOD MANUFACTURING: BACKGROUND 1

Section I: Principles and Establishments Classification 3

1. Fundamentals of Food Manufacturing 5
Wai-Kit Nip

2. Fermented Products and Their Manufacture 45
Wai-Kit Nip

3. Food Manufacturing in the United States: Standard Industrial Classification 85
Y.H. Hui

Section II: Flavors: Food Processing, Product Developments, and Recent Advances 117

4. Food Flavorings: Principles of Applications 119
Taiwo O. Omobuwajo

5. Product Development 131
Taiwo O. Omobuwajo

6. Extraction Modes 147
Marisa F. Mendes, Fernando L.P. Pessoa, Sílvio A.B. Vieira De Melo, and Eduardo M. Queiroz

7. Distillation and Drying 157
Fernando L.P. Pessoa, Marisa F. Mendes, Eduardo M. Queiroz, Sílvio A.B. Vieira de Melo, and David Lee Nelson

8. Genetic Engineering 169
Gláucia Maria Pastore and Gabriela Alves Macedo

9. Flavor Compounds Produced by Fungi, Yeasts, and Bacteria 179
Carlos R. Soccol, Adriane B.P. Medeiros, Luciana P.S. Vandenberghe, and Adenise L. Woiciechowski

10. Flavor Production by Solid and Liquid Fermentation 193
Carlos R. Soccol, Adriane B.P. Medeiros, Luciana P.S. Vandenberghe, and Adenise L. Woiciechowski

11. Herbs, Spices, and Essential Oils 205
Alain Darriet

Section III: Food Sanitation and Establishment Inspection 221

12. FDA GMPs, HACCP, and the Food Code 223
Nanna Cross

13. Food Establishment Inspection 245
Y.H. Hui

PART B: FOOD PRODUCTS MANUFACTURING 257

Section IV: Bakery Products 259

14. Manufacturing of Bread and Bakery Products 261
Weibiao Zhou and Nantawan Therdthai

15. Muffins and Bagels 279
Nanna Cross

16. Fundamentals of Cakes: Ingredients and Production 307
Frank D. Conforti

17. Traditional Italian Products from Wheat and Other Starchy Flours 327
M. Ambrogina Pagani, Mara Lucisano, and Manuela Mariotti

18. Flavor Migration in Solid Food Matrices 389
Yi-Chung Fu

Section V: Beverages 417

19. Carbonated Beverages 419
Daniel W. Bena

20. The Beer Brewing Process: Wort Production and Beer Fermentation 443
Ronnie Willaert

21. Manufacture of Whisky 507
Takefumi Yoneya

Section VI: Cereals: Rice and Noodles 521

22. Rice-Based Products 523
C.M. Rosell and C. Collar

23. Asian (Oriental) Noodles and Their Manufacture 539
Wai-Kit Nip

Section VII: Cheeses 565

24. Cheddar and Related Hard Cheeses 567
Stephanie Clark and Shantanu Agarwal

25. Pasteurized Process and Related Cheeses 595
Stephanie Clark and Shantanu Agarwal

26. Cottage Cheese 617
Stephanie Clark and David E. Potter

27. Cheese Varieties Made by Direct Acidification of Hot Milk 635
Ramesh C. Chandan

28. Cream Cheese as an Acidified Protein-Stabilized Emulsion Gel 651
Arjen Bot, Frank A.M. Kleinherenbrink, Michel Mellema, and Christel K. Magnani

Section VIII: Confectionery 673

29. Chocolate and Cocoa 675
Randall Hofberger and Nina Ann Tanabe

30. Confectionery: Inspection and Enforcement 695
Y.H. Hui

Section IX: Fats and Oils 703

31. Margarine and Dairy Spreads: Processing and Technology 705
Pernille Gerstenberg Kirkeby

32. Cream Products **725**
David W. Everett

33. Influence of Processing on Virgin Olive Oil Quality **751**
Maria Roca, Beatriz Gandul-Rojas, and M. Isabel Mínguez-Mosquera

Section X: Fruits and Fruit Juices **771**

34. Apple: Production, Chemistry, and Processing **773**
Nirmal K. Sinha

35. Strawberries and Blueberries: Phytonutrients and Products **793**
Nirmal K. Sinha

36. Major Tropical Fruits and Products: Banana, Mango, and Pineapple **815**
Lillian G. Po

37. Fruit Juices **847**
Terri D. Boylston

38. Dried Banana **867**
Raquel Pinho Ferreira Guiné and Maria João Barroca Dias

39. Fresh-Cut Fruits **879**
Olga Martín-Belloso, Robert Soliva-Fortuny, and Gemma Oms-Oliu

Section XI: Functional Foods **901**

40. Functional Foods and Ingredients **903**
Chung-Ja C. Jackson and Gopinadhan Paliyath

41. Functional Foods: International Considerations **939**
Kelley Fitzpatrick

42. Functional Foods Based on Dairy Ingredients **957**
Ramesh C. Chandan and N.P. Shah

43. Functional Properties of Milk Constituents **971**
Ramesh C. Chandan

44. Functional Foods Based on Meat Products **989**
Francisco Jiménez-Comenero

45. Gluten-Free Cereal Products as a Functional Food **1017**
Elke K. Arendt and Michelle M. Moore

VOLUME 1 INDEX **1037**

VOLUME 1 CONTENTS **1065**

VOLUME 2 CONTENTS **1069**

CUMULATIVE INDEX **1073**

Part A

Food Manufacturing: Background

This Part includes Sections I, II, and III.

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Section I

Principles and Establishments Classification

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

1

Fundamentals of Food Manufacturing

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1.1 Introduction and Goals 6
1.2 Food Spoilage and Foodborne Diseases 7
1.2.1 Food Spoilage 7
1.2.1.1 Food Spoilage and Biological Factors 7
1.2.1.2 Food Spoilage and Chemical (Including Biochemical) Factors 8
1.2.1.3 Food Spoilage and Physical Factors 9
1.2.1.4 Prevention and Retardation of Food Spoilage 10
1.2.1.5 Sources of Information 11
1.3 Product Formulations and Flow Charts 11
1.4 Units of Operations 13
1.4.1 Raw Materials Handling 20
1.4.2 Cleaning 21
1.4.3 Separating 22
1.4.4 Disintegrating 22
1.4.5 Forming 23
1.4.5.1 Meat and Poultry Patties 23
1.4.5.2 Pasta 23
1.4.5.3 Confectionery 23
1.4.6 Pumping 23
1.4.7 Mixing 24

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

1.5 Processing and Preservation Techniques 24
1.5.1 Heat Application 24
1.5.1.1 Why are Foods Heated? 25
1.5.1.2 Heat Exchangers for Liquid Foods 25
1.5.1.3 Tanks or Kettles for Liquid Foods 26
1.5.1.4 Pressure Cookers or Retorts for Packaged Foods 26
1.5.1.5 Roasters or Heated Vessels in Constant Rotation 26
1.5.1.6 Tunnel Ovens 26
1.5.2 Heat Removal or Cold Preservation 26
1.5.2.1 Chilling and Refrigeration Process 27
1.5.2.2 Freezing and Frozen Storage 29
1.5.2.3 Quality Changes in Food as a Consequence of Freezing and Frozen Storage 29
1.5.2.4 Types of Common Freezers with Different Cooling Media 30
1.5.3 Evaporation and Drying 31
1.5.4 Food Additives 32
1.5.4.1 Why are Additives Used in Foods? 33
1.5.4.2 What is a Food Additive? 34
1.5.4.3 What is a Color Additive? 35
1.5.4.4 How are Additives Regulated? 35
1.5.4.5 How are Additives Approved for Use in Foods? 36
1.5.4.6 Additional Information About Additives 36
1.5.5 Fermentation 38
1.5.6 New Technologies 38
1.5.6.1 Microwave and Radio Frequency Processing 39
1.5.6.2 Ohmic and Inductive Heating 39
1.5.6.3 High-Pressure Processing (HPP) 40
1.5.6.4 Pulsed Electric Fields (PEF) 40
1.5.6.5 High-Voltage Arc Discharge 41
1.5.6.6 Pulsed Light Technology 41
1.5.6.7 Oscillating Magnetic Fields 41
1.5.6.8 Ultraviolet Light 41
1.5.6.9 Ultrasound 42
1.5.6.10 Pulsed X-Rays 42
1.6 Packaging 42
General References 43
Specific References 44

1.1 INTRODUCTION AND GOALS

This chapter provides an overview of the basic principles of food manufacturing (processing). The goals of modern food manufacturing can be summarized as follows:

- *Formulation*. A logical basic sequence of steps to produce an acceptable and quality food product from raw materials.
- *Easy Manufacturing Procedures*. Development of methods that can facilitate the various steps of production.
- *Time Economy*. A cohesive plan that combines the science of production and manual labor to reduce the time needed to produce the product.

- *Consistency*. Application of modern science and technology to ensure the consistency of each batch of products.
- *Product and Worker Safety*. Close working relationship between government and manufacturers (processors), ensuring wholesome products for public consumption and a safe working environment for workers.
- *Buyer Friendly*. Assuming the buyer likes the product, the manufacturer must do everything humanly possible to ensure that the product is user friendly (size, cooking instruction, keeping quality, convenience, etc.).

Obviously, to achieve all the above goals is not a simple matter. This chapter is concerned mainly with the scientific principles of manufacturing safe food products. With this as a premise, the first question we can ask ourselves is the following: Why do we want to manufacture (process) food? At present, there are many *modern* reasons why foods are manufactured, for example, adding value to a food, improving the visual appeal, convenience. However, traditionally, the single most important reason that we wish to manufacture (process) food is to make it last longer without spoiling. Probably the oldest methods of achieving this goal are drying of cereals, fruits, and vegetables, salting of meat and fish, fermenting of milk, and pickling of vegetables. The next section discusses food spoilage and foodborne diseases.

1.2 FOOD SPOILAGE AND FOODBORNE DISEASES

1.2.1 Food Spoilage

Foods are made from natural materials, and, like any living matter, will deteriorate in time. The deterioration of food, or food spoilage, is the natural way of recycling, restoring carbon, phosphorus, and nitrogenous matters to the good Earth. However, spoilage (deterioration, putrefaction) will usually modify the quality of foods from good to bad, in the form of poor appearance (discoloration), offensive smell, and inferior taste. Food spoilage could be caused by a number of factors, chiefly biological, but also by chemical and physical factors. Consumption of spoiled foods can cause sickness, and even death. Thus, food safety is the major concern in spoiled foods.

1.2.1.1 Food Spoilage and Biological Factors. Manufactured (processed) and natural foods are composed mainly of carbohydrates, proteins, and lipids (fats and oils). The major constituents in vegetables and fruits are carbohydrates, including sugars (sucrose, glucose, and so on), polymers of sugars (starch), and other complex carbohydrates such as fibers. Lipids are the major components of oilseeds, milk and most cheeses, and proteins are the chief constituents of muscle foods. Under natural storage conditions, foods start to deteriorate once the living cells in the foods (of plant and animal origins) are dead or damaged. Secretion of internal proteases such as chymotrypsin and trypsin breaks up proteins at specific amino-acid positions, lipases and lyases from lyzosomes disintegrate the cells, de-esterificate fats (triglycerides) into fatty acids and glycerol, hydrolases hydrolyze proteins into amino acids, and starch into simpler sugars. The exposure of foods and damaged cells to the environment would attract microorganisms (e.g., bacteria, molds, and viruses), and insects, which in turn would further accelerate the decomposition of the food. Foods with contaminated microorganisms lead to foodborne illnesses, which, as reported by the Center for Disease Control and

TABLE 1.1 Most Common Bacteria Genera Found in Certain Food Types.

Microorganisms	Foods
Corynebacterium, Leuconostoc	Dairy products
Achromobacter	Meat, poultry, seafoods
Bacteriodes, Proteus	Eggs and meats
Pseudomonas	Meats, poultry, eggs

Prevention (CDC), causes approximately 76 million illnesses and 5000 deaths in the United States yearly (http//www.cdc.gov/foodsafety/). For most food poisoning, the spoilage has not reached the stage where the sensory attributes (appearance, smell, taste, texture, and so on.) of the food are abnormal.

Illness from food can be mainly classified as:

- Foodborne infection caused by pathogenic bacteria (disease-causing microorganisms, such as *Salmonella* bacteria, multiplying in a victim's digestive tract, causing diarrhea, vomiting, and fever, and so on), and
- Foodborne intoxication (food poisoning resulting from a toxin produced by pathogenic microorganisms, for example, *Clostridium botulinum* and *Staphylococcus aureus*, in the food itself).

Foodborne illness also has a major economic impact on society, costing billions of dollars each year in the form of medical bills, lost work time, and reduced productivity (McSwane and others 2003). Some genera of bacteria found in certain food types are listed in Table 1.1, and some common types of microorganisms found in foods are listed in Table 1.2. Some major bacterial and viral diseases transmitted to humans through foods are listed in Table 1.3. The interactive behavior of microorganisms may contribute to their growth and/or spoilage activity (Gram and others 2002).

1.2.1.2 Food Spoilage and Chemical (Including Biochemical) Factors. In many cases, when foods are oxidized, they become less desirable or are even rejected. The odor, taste, and color may change and some nutrients may be destroyed. Examples

TABLE 1.2 Most Common Pathogenic Bacteria and Viruses Found in Foods.

Bacteria	
Clostridium botulinum	*Listeria monocytogenes*
Salmonella spp.	*Staphylococcus aureus*
Clostridium perfringens	*Escherichia coli*
Botulinum spp.	*Campylobacter jejuni*
Streptococci spp.	*Bacillus cereus*
Lactobacillus spp.	*Proteus* spp.
Shigellas spp.	*Pseudomonas* spp.
Salmonella spp.	*Vibrio* spp.
Virus	
Hepatitis A virus	Echovirus
Rotavirus	Calcivirus

TABLE 1.3 Some Major Bacterial and Viral Diseases Transmitted to Humans Through Food.

Bacteria	Diseases
Campylobacter jejuni	Campylobacteriosis
Enterobacteriaceae	Enteric disease
Listeria monocytogenes	Listeriosis
Salmonella spp.	Salmonellosis
Salmonella typhi	Typhoid fever
Shigella dysenteriae	Dysentery
Vibrio cholerae	Cholera
Yersinia enterocolitica	Diarrheal disease
Virus	**Diseases**
ECHO virus	Gastroenteritis
HAV virus	Hepatitis type A
Norwalk agent	Viral diarrhea
Rotavirus	Infant diarrhea

are the darkening of the cut surface of a potato and the browning of tea color with time. Oxidative rancidity results from the liberation of odorous products during breakdown of unsaturated fatty acids. These products include aldehydes, ketones, and shorter-chain fatty acids.

Browning reactions in foods include three nonenzymatic reactions (Maillard, caramelization, and ascorbic acid oxidation), and one enzymatic reaction (phenolase browning) (Fennema 1985). Heating conditions in the surface layers of food cause the Maillard browning reaction between sugars and amino acids, for example, in the browning of baked bread and cakes. The high temperatures and low moisture content in the surface layers also cause caramelization of sugars, and oxidation of fatty acids to other chemicals such as aldehydes, lactones, ketones, alcohols, and esters (Fellows 1992). Moisture and heat can also produce hydrolytic rancidity in fats, in this case fats are split into free fatty acids, which may cause off-odors and rancid flavors in fats and oils (Potter and Hotchkiss 1995). Thermal decomposition of ascorbic acid under both aerobic and anaerobic conditions, by oxidative or nonoxidative mechanisms, in either the absence or presence of amino compounds, can also cause browning (Wedzicha and McWeeny 1974). The formation of ripening fruit flavor often results from Strecker degradation (the transamination and decarboxylation) of amino acids, such as the production of 3-methylbutyrate (apple-like flavor) from leucine (Drawert 1975). Further heating of the foods can break down some of the volatiles generated by the Maillard reaction and Strecker degradation to produce burnt or smoky aromas associated with browning. Enzymatic browning occurs on cut surfaces of light-colored fruits (apples, bananas) and vegetables (potatoes), due to the enzymatic oxidation of phenols to orthoquinones, which in turn rapidly polymerize to form brown pigments known as melanins.

1.2.1.3 Food Spoilage and Physical Factors. Food spoilage can also be caused by physical factors, such as temperature, moisture, and pressure acting upon the foods. Moisture and heat can also produce hydrolytic rancidity in fats (mentioned earlier), with production of off-odors and rancid flavors in fats and oils as mentioned earlier (Potter and Hotchkiss 1995). Excessive heat denatures proteins, breaks emulsions, removes moisture from food, and destroys nutrients such as vitamins. However, excessive coldness, such as freezing, changes their texture and/or cracks their outer coatings to permit contamination by microorganisms. Foods under pressure will be squeezed and transform

into unnatural conformation. The compression will likely break up the surface structure (cracking of eggs), release degradative enzymes (bruising of fruits such as apples and pears), and expose the damaged food to exterior microbial contamination.

Of course, many health officials consider physical factors to include such things as sand, glass, wood chip, rat hair, animal urine, bird droppings, insect parts, and so on. They may not spoil the food, but they can present health hazards. Some of these foreign substances do lead to spoilage. Insects and rodents can consume and damage stored foods, and insects can lay eggs and leave larvae in the foods, causing further damage later. Such foods are no longer reliable because they contain hidden contaminants. The attack of foods by insects and rodents can also contaminate foods further with microbial infections.

1.2.1.4 Prevention and Retardation of Food Spoilage. Food spoilage can be prevented by proper sanitary practices in food handling and processing, appropriate preservation techniques, and standardized storing conditions.

Food Handling and Processing. The entire process, from raw ingredients to a finished product for storage, must comply with a standard sanitation program. In the United States, the practice of HACCP (hazard analysis critical control points), mandatory at present for several industries, may eventually become so for all food industries. At present, the application of HACCP is voluntary for most food processors. Similar sanitary programs apply to workers. It is important to realize that a food processing plant must have a basic sanitation system program before it can implement a HACCP program.

Food Preservation. There are many techniques used to preserve food, such as legal food additives, varying levels of food ingredients or components, traditional and new technologies. Legal food additives, among other functions, can prevent oxidation and inhibit or destroy harmful microorganisms (molds and bacteria). Vitamin E or vitamin C can serve as antioxidants in many food products and benzoate in beverages can act as an antimicrobial agent. We can preserve food by manipulating the levels of food ingredients or components to inhibit the growth of microorganisms or destroy them, for example, by keeping the food low in moisture content (low water activity), high in sugar or salt content, or at a low pH (less than pH 5). Other traditional technologies such as canning (thermal processing), fermentation, refrigeration, and freezing are well-established preservation methods. Recently, new or alternative technologies have become available to preserve food. Because they are new, their applications are carefully monitored. Nothing in the last two decades seems to have generated more publicity than the use of X-rays in food processing. Although food irradiation has been permitted in the processing of several categories of food, its general application is still carefully regulated in the United States.

Food Packaging and Storage. Raw and processed foods should be packaged to prevent oxidation and microbial contamination, insect infestation, and loss of moisture and integrity. Storage of foods (when not contaminated) below $-20^{\circ}C$ can allow food to be kept for several months or a year. Foods stored at $4^{\circ}C$ can have their shelf-life extended to several days or a week (note that some bacteria such as *Listeria monocytogenes* can still grow and multiply even in foods at refrigerated temperatures).

Newly developed techniques to preserve foods include the incorporation of bacteriocin in plastic to retain activity and to inhibit surface growth of bacteria on meat (Siragusa and others 1999), and the application of an intelligent Shelf Life Decision System for quality optimization of the food chill chain (Giannakourou and others 2001).

1.2.1.5 Sources of Information. At present, all major Western government authorities have established Web sites to educate consumers and scientists on the safe processing of food products. Internationally, two major organizations have always been authoritative sources of information: World Health Organization (WHO) and Food and Agriculture Organization (FAO). They also have comprehensive Web sites.

In the United States, major federal authorities on food safety include, but are not limited to the following:

- Department of Agriculture (USDA),
- Food and Drug Administration (FDA),
- Centers for Disease Control (CDC),
- Environmental Protection Agency (EPA),
- National Institutes of Health (NIH).

Many trade associations in Western countries have Web sites that are devoted entirely to food safety. Some examples in the United States include

- American Society of Microbiologists,
- Institute of Food Technologists,
- International Association of Food Protection,
- National Food Processors Association,
- Food Products Association,
- National Restaurants Association.

All government or trade association Web sites are easily accessible by entering the agency name into search engines.

1.3 PRODUCT FORMULATIONS AND FLOW CHARTS

As mentioned earlier, for many food products, processing is an important way to preserve the product. However, for some food products, many self-preserving factors play a role, such as ingredients and their natural properties. Three good examples are pickles, barbecue sauces, and hard candies. Preserving pickles is not difficult if the end product is very sour (acidic) or salty. Traditionally, barbecue sauces have a long shelf-life because of the high content of sugar and acids. Most unwrapped hard candies keep a long time, assuming the environment is at room temperature and not very humid. Most wrapped hard candies last even longer if the integrity of the wrappers is maintained. For baked products (cookies, bread), measures against spoilage take second place to consumer acceptance of freshness. So, the objectives of processing of foods vary with the product. However, one aspect is essential to all manufacturers, as discussed below.

To manufacture a food product, it is assumed that the manufacturer (processor) has a formula for the product. In countries all over the world, small family-owned food businesses usually start with home recipes for popular products instead of a scientific formula. Most of us are aware of such humble beginnings of major corporations manufacturing cola (carbonated), soft drinks, cheeses, breakfast cereals, and many others. When these family businesses were started, there was not much science and technology involved.

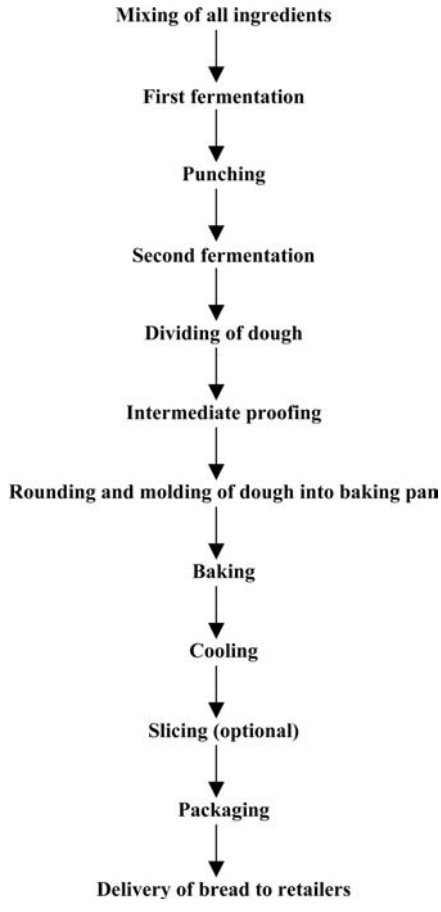

Figure 1.1 *A general flow chart for the manufacture bread.*

As the companies grew and had many employees, they started to hire food scientists, food technologists, and food engineers to study the "recipe(s)" and refine every aspect of the product until the entire manufacturing process was based on sound scientific, technical, and engineering principles. After that, all efforts were directed towards production and marketing. Even now, somewhere a person will start making "barbecue sauce" in his garage and selling it to his neighbors. Although very few of these starters will succeed, this trend will continue to be the case in view of the free enterprise spirit of the West.

Although any person can start manufacturing food using a home recipe, the federal government in the United States has partial or total control over certain aspects of the manufacturing processes for food and beverage products. This control will automatically affect the recipes, formulas, or specifications of the products. Although the word "control" here refers mainly to safety, it is understood that it will affect the formulations to some extent, especially critical factors such as temperature, pH, water activity, and so on.

Flow charts differ from formula in that they provide an overview of the manufacturing process. For illustration, Figures 1.1 to 1.8 provide examples of flow charts for the manufacture of bakery (bread), dairy (yogurt), grain (flour), fruits (raisins), vegetables (pickles), meat (frankfurters, frozen chicken parts), and seafood (canned tuna).

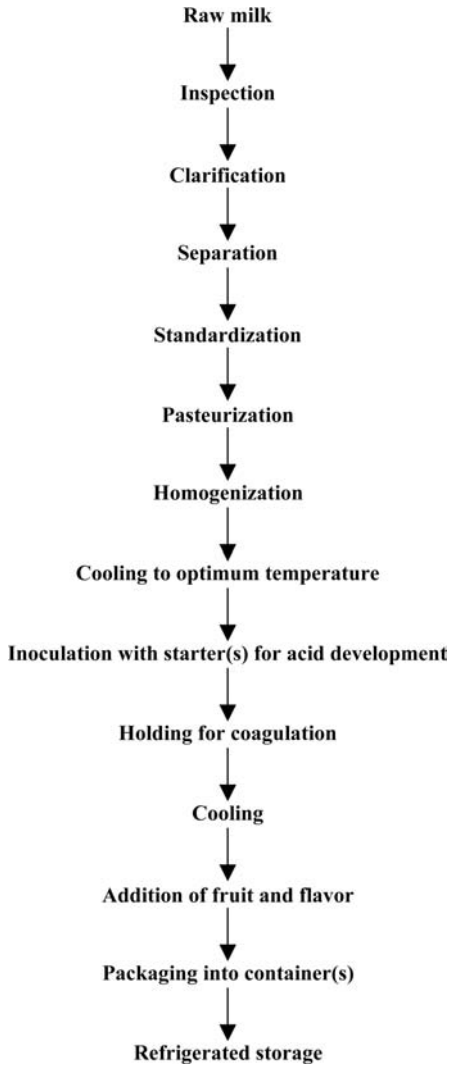

Figure 1.2 *A general flow chart for the manufacture of Yogurt.*

1.4 UNITS OF OPERATIONS

The manufacturing (processing) of most food products involves many of the following unit operations:

- Raw materials,
- Cleaning,
- Separating,
- Disintegrating,
- Forming, raw,
- Pumping,

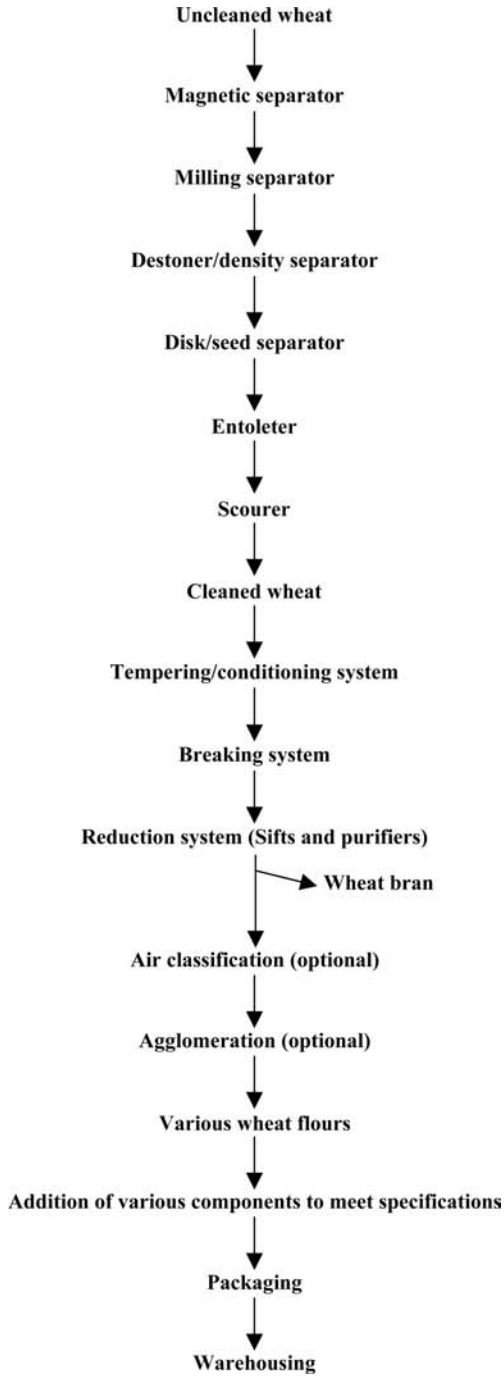

Figure 1.3 *A general flow chart for the production of flour from wheat.*

Figure 1.4 *A general flow chart for the production of raisins.*

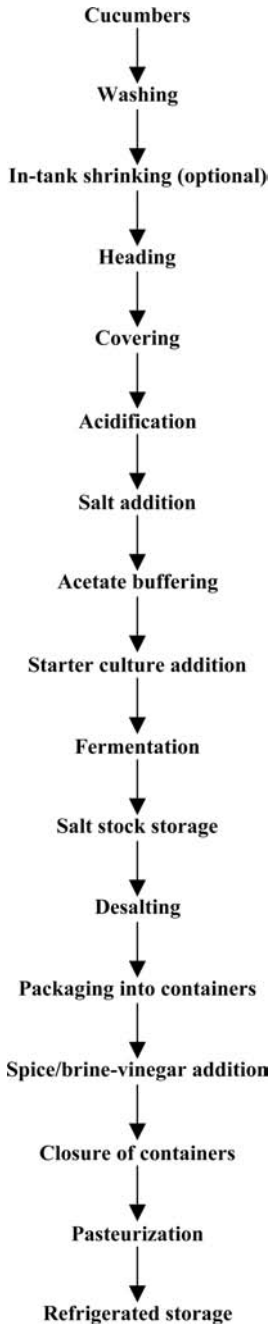

Figure 1.5 *A general flow chart for the production of pickles.*

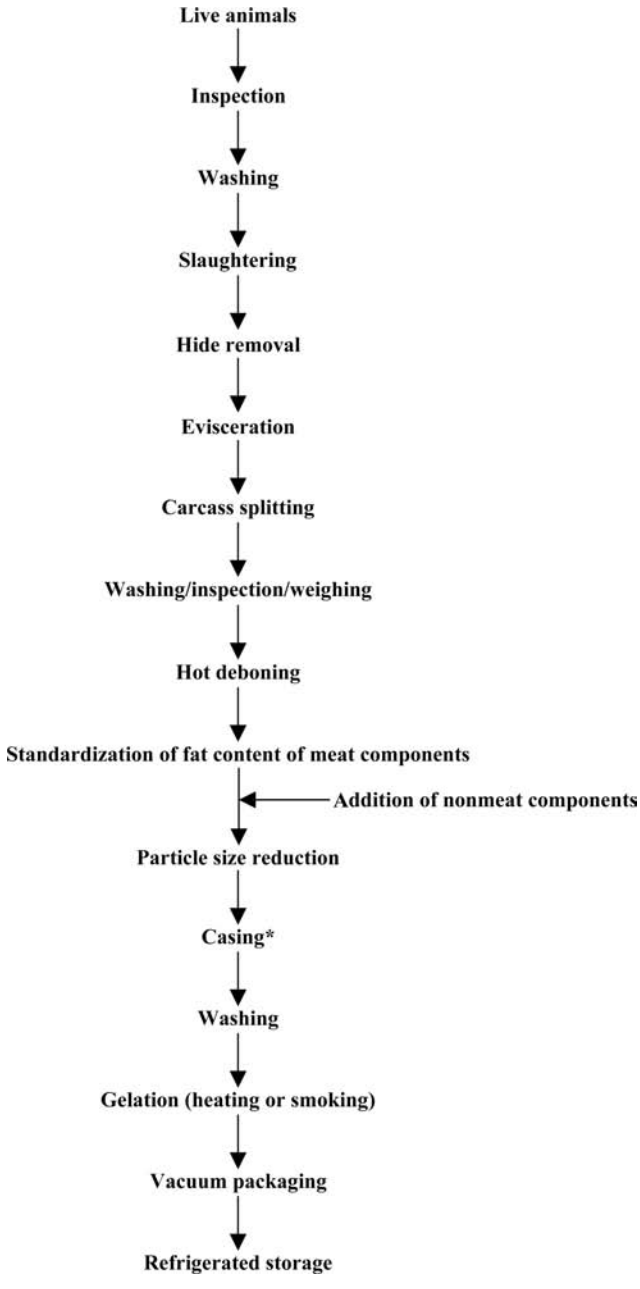

Figure 1.6 *A general flow chart for the production of Frankfurters.*

- Mixing,
- Application methods (formulations, additives, heat, cold, evaporation, drying, fermenting, and so on),
- Combined operations,
- Forming, finished product.

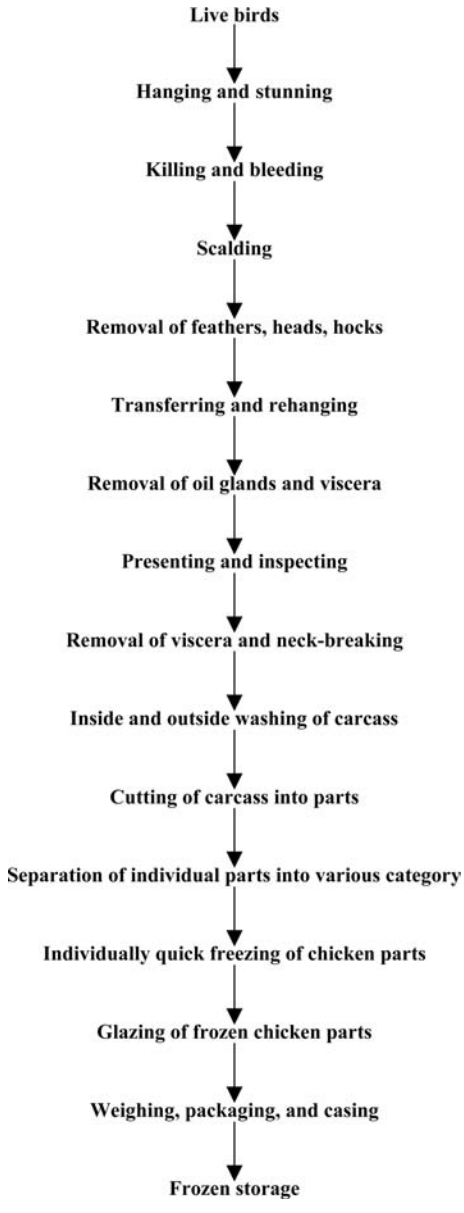

Figure 1.7 *A general flow chart for the production of frozen chicken parts.*

Instead of classifying the following as units of operations, we will discuss them and similar procedure as separate topics:

- Heating,
- Cooling,
- Sanitation,
- Quality control,
- Packaging.

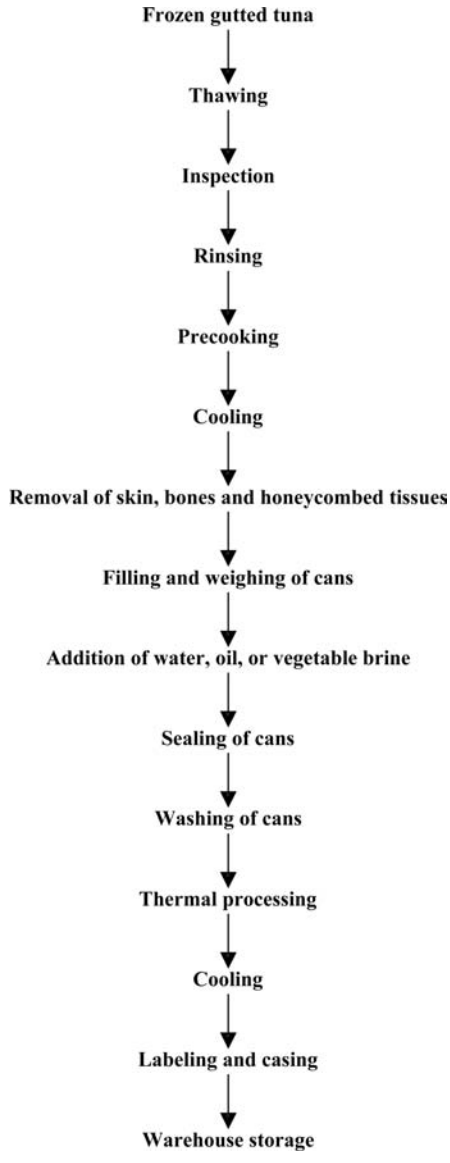

Figure 1.8 *A general flow chart for the production of canned tuna.*

According to the United States Department of Labor, there are hundreds of different categories of food products being manufactured. Correspondingly, there are hundreds of companies manufacturing each category of food product. In sum, there are literally thousands of food manufacturers. Two major reasons for this explosion of new companies are the constant introduction of new products and improvement in manufacturing methods and equipment.

To facilitate the technological processing of food at educational and commercial levels, food manufacturing (processing) professionals have developed unified principles and a systematic approach to the study of these operations. The involved processes of the food industry can be divided into a number of common operations, termed unit operations. Depending on the manufacturer (processor), such unit operations vary in name and

number. For ease of discussion, we use the following unit operations (in alphabetical order) for the most common ones:

- Cleaning,
- Coating,
- Controlling,
- Decorating,
- Disintegrating,
- Drying,
- Evaporating,
- Forming,
- Heating,
- Mixing,
- Packaging,
- Pumping,
- Raw materials handling,
- Separating.

During food processing, the manufacturer selects and combines unit operations into unit processes, which are then combined to produce more complex and comprehensive processes. Although emerging technology plays an important role in food processing as time progresses, these units, in the order they appear in a food processing plant, still apply.

1.4.1 Raw Materials Handling

Raw materials are handled in various ways, including:

- Hand and mechanical harvesting on the farm;
- Trucking (with or without refrigeration) of fruits and vegetables;
- Moving live cattle by rail; and
- Conveying flour from transporting vehicle or boats to storage bins.

Some good examples are as follows.

- Oranges are picked on the farm by hand or mechanical devices, moved by truck trailers, usually refrigerated, to juice processing plants, where they are processed. Of course, the transport must take into account the size of the trucks, length of time during transport, and temperature control. The major objective is to avoid spoilage. In recent years, the use of modified atmosphere packaging/storage has increased the odds in favor of the farmers and producers.
- Handling sugar and flour poses great challenges. When dry sugar reaches the processing plants, via truck trailers or rail, it is transported to storage bins via a pneumatic lift system. The sugar will cake if the storage time, temperature, and humidity are not appropriate. Improper transfer of sugar may result in dusting and buildup of static electricity, which can cause an explosion, because sugar particles are highly combustible. The same applies to finely divided flour.

In handling raw materials, one wishes to achieve the following major objectives:

- Proper sanitation;
- Minimal loss of product;
- Acceptable product quality;
- Minimal bacterial growth,
- Minimal holding time.

1.4.2 Cleaning

We all know what cleaning a raw product means. Before we eat a peach, we rinse it under the faucet or with potable water. Before we make a salad, we wash/rinse the vegetables with potable water. Before we eat crabs, we clean them. Of course, the difference in cleaning between home kitchens and a food processing plant is the volume. We clean one peach; they clean a thousand peaches.

Depending upon the product and the nature of the dirt, cleaning can be accomplished with the following methods or devices, individually or in combination:

- Air (high-velocity),
- Brushes,
- Magnets,
- Steam,
- Ultraviolet light,
- Ultraviolet sound,
- Vacuum,
- Water.

There are other new technologies that will not be discussed here.

Water is probably the most common cleaning agent and its applications vary. Examples include the following:

- Clams, oysters, crabs, and other shellfish are commonly hosed to remove mud, soil, and other foreign debris. If they are contaminated, they may have to be purged by incubating them in running clean seawater.
- City water will not be acceptable for manufacturing beverages. It will have to be further treated with chemical flocculation, sand filtration, carbon purification, microfiltration, deaeration, and so on. This is not considered a simple cleaning. Rather it is a process in cleaning.
- Eviscerating poultry can be considered a cleaning operation if we make use of water. However, the actual process of removing the entrails may involve vacuuming in addition to water.
- With a product like pineapple, the irregular surface is usually cleaned by the scrubbing action of high-pressure water jets.

Just as in a home kitchen where pots and pans require frequent cleaning, state and federal regulations require that the equipment used in a food processing plant be

cleaned and sanitized after each use. After dirt and mud are removed, some raw products also require special sanitizing procedures. The use of sanitizers can be a complicated matter involving different types of sanitizers, federal regulations, expertise, and so on.

1.4.3 Separating

In food processing, the step of separating may involve separating

- A solid from a solid, as in peeling of potatoes;
- A solid from a liquid, as in filtration;
- A liquid from a solid, as in pressing juice from a fruit;
- A liquid from a liquid, as in centrifuging oil from water;
- A gas from a solid or a liquid, as in vacuum canning.

One of the time-honored techniques in the separating operation is the hand sorting and grading of individual units, such as mushrooms, tomatoes, oranges. Many mechanical and electronic sorting devices have now replaced human hands in handling various types of raw food products. An electronic eye can identify differences in color as the products are going along the conveyor belt. Built-in mechanisms can sort the products by color, into "good" versus "bad" color. The current invention of the electronic nose also shows promises, and automatic separation according to size is easily accomplished by passing fruits or vegetables over differently sized screens, holes, or slits.

1.4.4 Disintegrating

Disintegrating means subdividing large masses of foods into smaller units or particles. This may include

- Cutting;
- Grinding;
- Pulping, homogenizing; or
- Other methods.

Examples include

- Automatic dicing of vegetables;
- Mechanical deboning of meat;
- Manual and automatic cutting of meat into wholesale and retail sizes;
- Cutting of bakery products with electric knives and other products with water jets (high-velocity and high-pressure);
- Disintegrating various categories of food products with high-energy beams and laser beams;

- Homogenization using commercial blenders, high-pressure traveling through a valve with very small openings, ultrasonic energy, and so on. Homogenization is probably one of the most important stages (if not the most important) in dairy manufacturing. Homogenization produces disintegration of fat globules in milk or cream from large globules and clusters into minute globules. This is done by forcing the milk or cream under high pressure through a valve with very small openings.

1.4.5 Forming

Forming is an important operation in manufacturing many foods:

- Meat and poultry patties;
- Confections such as candies, jelly beans, fruit juice tablets;
- Breakfast cereals;
- Pasta;
- Kinds such as some cheese cubes, processed cheese slices, potato chips, and others.

1.4.5.1 Meat and Poultry Patties. Patty-making machines are responsible for making ground meat and poultry patties by gently compacting the product into a disk shape. Uniform pressure is applied to produce patties with minimal variation in weight. Excessive pressure may result in tough cooked patties.

1.4.5.2 Pasta. Pasta products are formed by forcing dough through extrusion dies of various forms and shapes before drying in an oven.

1.4.5.3 Confectionery. The shapes and forms in the confectionery industry, such as candies and jellies, are made in several ways. Two of the most popular methods are the use of molds and cutting:

- *Molds*. The traditional use of molds is applied in making confectionery such as fondants, chocolate, and jellies. The product is deposited into molds to cool and harden.
- *Cutting*. Hard candies and toffee are cut into pieces after kneading or pulling.

1.4.6 Pumping

In food processing, pumping moves food (liquid, semi-solid, paste, or solid) from one step to the next or from one location to another. There are many types of pumps available, some with general, others specialized, applicability. The type of pump used depends on the food (texture, size, etc.). For example, broth, tomato pastes, ground meat, corn kernels, grapes, and other categories of food all require a “different” pump to do the job. Two of the most important properties of pumps are the ability to break up foods, and ease of cleaning.

1.4.7 Mixing

The operation of mixing, for example, includes

- Kneading,
- Agitating,
- Blending,
- Emulsifying,
- Homogenizing,
- Diffusing,
- Dispersing,
- Stirring,
- Beating,
- Whipping, and
- Movements by hands and machines.

Examples of mixing include

- Homogenization to prevent fat separation in milk;
- Mixing and developing bread dough, which requires stretching and folding, referred to as kneading;
- Beating in air, as in making an egg-white foam;
- Blending dry ingredients, as in preparing a ton of dry cake mix;
- Emulsifying, as in the case of mayonnaise.

Commercial mixers for food processing come in many shapes and forms because many types of mixtures or mixings are possible. Two examples are provided as illustration:

1. *Mixing solids with solids*, for example, a dry cake mix. The mixer must cut the shortening into the flour, sugar, and other dry ingredients in order to produce a fluffy homogeneous dry mix. A ribbon blender is used.
2. *Beating air into a product while mixing*, for example, using a mixer-beater in an ice-cream freezer. The mixer turns in the bowl in which the ice-cream mix is being frozen. This particular operation permits the mixer to achieve several tasks or objectives: it beats air into the ice-cream to give the desired volume and overrun, and it keeps the freezing mass moving to produce uniformity and facilitate freezing.

1.5 PROCESSING AND PRESERVATION TECHNIQUES

1.5.1 Heat Application

Heat exchanging, or heating, is one of the most common procedures used in the manufacture of processed foods. Examples include pasteurizing of milk, manufacturing of bakery products, roasting peanuts, and canning. Foods may be heated or cooked using

- Direct injection of steam,
- Direct contact with flame,

- Toasters,
- Electronic energy as in microwave cookers, and
- Many new forms of technology.

Whatever the method, precise control of temperature is essential. Heating is used in

- Baking,
- Frying,
- Food concentration,
- Food dehydration, and
- Package closure.

1.5.1.1 Why are Foods Heated? All of us know why we cook food at home: to improve texture, to develop flavors, to facilitate mixing of water, oil, and starch, to permit caramelization, and so on. Commercially, the basic reasons for heating are simple and may include the following:

1. Destruction of microorganisms and preservation of food (food canning and milk pasteurization are common examples);
2. Removal of moisture and development of flavors (ready-to-eat breakfast cereals and coffee roasting are common examples);
3. Inactivation of natural toxicants (processing soy-bean meal is a good example);
4. Improvement of the sensory attributes of the food such as color, texture, and mouth-feel;
5. Combination of ingredients to develop unique food attributes and to attract consumer preferences.

Traditional thermal processing of foods uses the principles of transferring heat energy by conduction, convection, radiation, or a combination of these. Foods are heated in a variety of traditional equipment by applying basic food engineering principles using heat exchangers, tanks or kettles, retorts, and toasters. Other methods may include direct injection of steam, and direct contact with flame. Heating food with electronic energy (microwave) is a relatively new technique. Later in this chapter, other new technologies for heating foods will be discussed.

1.5.1.2 Heat Exchangers for Liquid Foods. As foods are sensitive to heat, special consideration is needed. Dark color, burnt flavors, and loss of nutrients can result from heating, especially prolonged heating. Heat exchangers have special advantages. They permit

1. Liquid food to have a maximal contact with the heat source and
2. Rapid heating and cooling.

For example, a plate-type heat exchanger is used to pasteurize milk. The equipment is composed of many thin plates. When milk flows through one side of the plates, it is heated by hot water on the other side. This provides maximal contact between the heat source and milk, resulting in rapid heating in a short time. The cooling is the reverse process after the milk has been heated. Instead of hot water, cooling water or brine is used.

1.5.1.3 Tanks or Kettles for Liquid Foods. During heating, hot water or pressurized steam circulating in the jackets of the tanks or kettles heats the food, and the reverse for cooling. This technique works for full liquid foods or partially liquid foods such as soups.

1.5.1.4 Pressure Cookers or Retorts for Packaged Foods. The most common method of sterilizing canned foods uses pressure cookers or retorts. Beginning in the early 1970s, the risk of botulism in canned food with low acidity prompted the U.S. FDA to implement stringent regulations governing this group of foods. Although the name of Hazards Analysis Critical Control Points (HACCP) did not have wide usage at the time, the regulations governing the production of low-acid canned foods can be considered the earliest form of HACCP program. Large pressure cookers or retorts are used to ensure that the canned goods are heated above the boiling point of water. The high temperature is generated by steam under pressure in a large retort designed to withstand such pressure and temperature. In this case, convection and conduction of heat energy are achieved. Steam hits the outside of the cans and energy is conducted into the can. Some form of moving or agitating device permits improved convection to occur inside the cans. Although there are other modern techniques of heating canned food products, many smaller companies still depend heavily on these traditional methods.

1.5.1.5 Roasters or Heated Vessels in Constant Rotation. Instead of one or two pieces of equipment, this system contains several units: loading containers, conveyor belts, hoppers, vats or vessels. The vessels are usually cylindrical in shape with built-in heating devices. Heat is generated via one of the following methods:

1. Circulation of heated air, which heats the food products inside the vessels;
2. Application of direct heat to the outside of the vessel by means of steam, flame (gas), and air (hot), for example, whereby heat is radiated from the inside walls of the vessels to the food.

The above unit system is best for roasting coffee beans or nuts.

1.5.1.6 Tunnel Ovens. This can be used for a variety of food products. The product is placed on a conveyor belt, which moves under a heat source. Sometimes, the product is vibrated so that heat distribution is even. Temperature control is essential, enabling products such as coffee beans or nuts to be roasted using this method.

1.5.2 Heat Removal or Cold Preservation

Cold preservation is a preservation method achieved by the removal of heat. It is among the oldest methods of preservation. Since 1875, with the development of mechanical ammonia refrigeration systems, commercial refrigeration and freezing processes have been available. A reduction in the temperature of a food reduces the rate of quality changes during storage caused by a number of factors. At low temperatures, microbial growth is retarded and its reproduction prevented. The rate of chemical reactions (e.g., oxidation, Maillard browning, formation of off-flavor), biochemical reactions (e.g., glycolysis, proteolysis, enzymatic browning, and lipolysis), and physical changes between

food components and the environment (e.g., moisture loss in drying out of vegetables) can also be reduced.

Most food spoilage organisms grow rapidly at temperatures above 10°C, although some grow at temperatures below 0°C, as long as there is unfrozen water available. Most pathogens do not grow well at refrigeration temperatures, except some psychrophilic bacteria such as *Listeria monocytogenes*, which commonly grows in dairy products. Below −9.5°C, there is no significant growth of spoilage or pathogenic microorganisms.

In general, the longer the storage period, the lower the temperature required. Pretreatment with intensive heat is not used in this process operation and, together with adequate control over enzymatic and microbiological changes, the food maintains its nutritional and sensory characteristics close to fresh status and results in a high-quality product. In comparing chilled and frozen foods, chilled food has a higher quality but a shorter shelf-life, and frozen food has a much longer shelf-life, but the presence of ice in the frozen product may contribute some undesirable changes in food quality.

1.5.2.1 Chilling and Refrigeration Process. The chilling process is the gentlest method of preservation, generating the least significant changes in taste, texture, nutritive value, and other attributes of foods. Generally it refers to a storage temperature above freezing, between about 16°C and −2°C. Most foods do not freeze until −2°C or slightly lower because of the presence of solutes such as sugars and salts. Commercial and household refrigerators usually operate at between 4.5°C and 7°C.

In low-acid chilled foods, strict hygienic processing and packaging areas required to ensure food safety. The chilling process is usually used in combination with other preservation methods such as fermentation, irradiation, pasteurization, mild heat treatment, chemicals (acids or antioxidants), and controlled atmosphere. The combination of these methods avoids the extreme conditions that must otherwise be used to limit microbial growth, thus providing a high-quality product (e.g., marinated mussels and yogurt).

Not all foods can be stored under chilled conditions. Tropical and subtropical fruits suffer chilling injury when stored below 13°C, resulting in abnormal physiological changes: skin blemishes (e.g., banana), browning in the flesh (e.g., mango), or failure to ripen (e.g., tomato). Some other foods should not be refrigerated; for example, breads stale faster at refrigeration temperature than at room temperature. Starch in puddings also tends to retrograde and result in syneresis.

There are several important considerations in producing and maintaining high-quality chilled foods.

1. *Quick Removal of Heat at the Chilling Stage*. Ideally, refrigeration of perishable foods starts at the time of harvest, slaughter, or at the finishing production line. Cooling can be accelerated by the following techniques.
   a. *Evaporative cooling*: Spray water and then subject food to vacuum (e.g., leafy vegetables).
   b. *Cryogenic cooling*: Nitrogen gas from evaporating liquid nitrogen, dry ice, and liquid carbon dioxide are used to remove heat for different products.
   c. *Heat exchangers*:
      i. Thin plates where warm bulk liquid foods pass chilled or "super-chilled" cooling fluid, separated by a thin stainless steel plate.
      ii. Cooling coils for liquid food.

2. *Maintaining Low Temperature During the Chill Storage*. This can be affected by the following factors:
   a. *Refrigeration design* (i.e., cooling capacity and insulation): This must be taken into account because temperature can be affected by heat generated by lights and electric motors, people working in the area, the number of doors and how they are opened, and the kinds and amounts of food products stored.
   b. *Refrigeration load*: Quantity of heat that must be removed from the product and the storage area in order to decrease from an initial temperature to the selected final temperature and then to maintain this temperature for a specific time.
   c. *Types of food*:
      i. *Specific heat of food*: Quantity of heat that must be removed from a food to lower it from one temperature to another. The rate of heat removal is largely dependent on water content.
      ii. *Respiration rate of food*: Some foods (fruits and vegetables) respire and produce their own heat at varying rates. Products with relatively high respiration rates (snap beans, sweet corn, green peas, spinach, and strawberries) are particularly difficult to store.
3. *Maintaining Appropriate Air Circulation and Humidity*. Proper air circulation helps to move heat away from the food surface toward refrigerator cooling coils and plates. Air velocity is important, especially in commercial coolers or freezers, in maintaining the appropriate relative humidity (RH), because if the RH is too high, condensation of moisture on the surface of cold food may occur, thus causing spoilage through microbial growth or clumping of the product. However, if RH is too low, dehydration of food may occur instead. Therefore, it is important to control the RH of the cooler and have proper packaging of food.
4. *Modification of Gas Atmosphere*. Chilled storage of fresh commodities is more effective if it is combined with control of the composition of the storage atmosphere. A reduction in oxygen concentration and/or increase in carbon dioxide concentration of the storage atmosphere reduces the rate of respiration (and thus maturation) of fresh fruits and vegetables, and also inhibits rates of oxidation, microbial, and insect growth. The atmospheric composition can be changed using three methods:
   a. *Controlled Atmosphere Storage* (*CAS*), where the concentration of oxygen, carbon dioxide, and ethylene are monitored and regulated throughout the storage. It is used to inhibit overripening of apples and other fruits in cold storage. Stored fruit and vegetables consume O_2 and give off CO_2 during respiration.
   b. *Modified Atmosphere Storage* (*MAS*), where the initially modified gas composition in a sealed storage is allowed to change by normal respiration of the food, but little control is exercised. The O_2 is reduced but not eliminated, and CO_2 is increased (the optimum levels differ for different fruits).
   c. *Modified Atmosphere Packaging* (*MAP*), where the fruit and vegetables are sealed in a package under flushed gas (N_2 or CO_2), and the air in the package is modified over time by the respiring product. Fresh meat (especially red meats) is packaged similarly.
5. *Efficient Distribution Systems*. To supply high-quality chilled foods to consumers, a reliable and efficient distribution system is also required. This involves chilled stores, refrigerated transportation, and chilled retail display cabinets. It requires careful control of the storage conditions, as above.

1.5.2.2 Freezing and Frozen Storage. Freezing is a unit operation in which the temperature of a food is reduced below the freezing point and a proportion of the water undergoes a phase change to form ice. Proper freezing preserves foods without causing major changes in their shape, texture, color, and flavor. Good frozen storage requires temperatures of $-18^{\circ}C$ or below; however, it is cost prohibitive to store lower than $-30^{\circ}C$. Frozen foods have increased in their share of sales since freezers and microwaves have become more available.

The major commodities commonly frozen are

- Fruits (berries, citrus, and tropical fruit), either whole, pureed, or as juice concentrate;
- Vegetables (peas, green beans, sweet corn, spinach, broccoli, Brussels sprouts, and potatoes such as French fries and hash browns);
- Fish fillets and seafoods, including fish fingers, fish cakes, shellfish, and prepared dishes with sauces;
- Meats (beef, lamb, and poultry) as carcasses, boxed joints, or cubes, and meat products (sausages and beef burgers);
- Baked goods (bread, cakes, pastry dough, and pies);
- Prepared foods (pizzas, desserts, ice cream, and dinner meals).

Principles of Freezing. The freezing process implies two linked processes: lowering of temperature by the removal of heat and a change of phase from liquid to solid. The change of water into ice results in an increase in concentration of the unfrozen matrix and therefore leads to dehydration and lowering of water activity. Both the lowering of temperature and water activity contribute to freezing as an important preservation method.

In order for a product to freeze, the product must be cooled below its freezing point. The freezing point of a food depends on its water content and the type of solutes present. The water component of a food freezes first and leaves the dissolved solids in a more concentrated solution, which requires a lower temperature to freeze. As a result, the freezing point decreases during freezing as the concentration increases. Different solutes depress the freezing point to a different degree.

Rate of Freezing. Faster freezing produces small crystals, necessary for high-quality products such as ice-cream. There are two main opposing forces affecting the freezing rate. The driving force includes the difference in temperatures between the freezing medium and the product; the bigger the difference, the faster the product will cool down. High thermal conductivity of the freezing medium (the efficiency with which the refrigerating agent extracts heat) and direct surface contact between medium and product also help to freeze the product quickly. On the other hand, the resistance force to quick freezing includes the product being packed in big sizes, irregular product geometry affecting direct contact with the freezing agent, and the product composition having a high heat capacity. Thermal conductivity of food packages such as cardboard and plastics will act to insulate heat transfer and thus slow down the freezing rate.

1.5.2.3 Quality Changes in Food as a Consequence of Freezing and Frozen Storage. As a consequence of the formation of ice, some negative changes in the quality of food result. The two major causes are freeze concentration effect and ice recrystallization.

Effect of Concentration of Solutes on Food Quality. The quality of products will change if solutes in the frozen product precipitate out of solution, for example, leading to a loss of consistency in reconstituted frozen orange juice because of aggregated pectic substances, and syneresis of starch pudding because of starch aggregation. The increase in ionic strength can lead to "salting out" of proteins, causing protein denaturation (a reason for the toughening of frozen fish). An increase in solute concentration may lead to the precipitation of some salts, and the anion/cation ratio of colloidal suspensions would then be disturbed and causes changes in pH. Such changes would also cause precipitation of proteins and changes in color of anthocyanin in berries. The concentration of solutes in the extracellular fluid will cause dehydration of adjacent tissues in fruit and vegetables, which would not be able to rehydrate after thawing. Lastly, the concentration of reactive compounds would accelerate reactions such as lipid oxidation.

Large Ice Crystal and Recrystallization Damage. If the food is not stored under sufficiently cold and steady temperatures, ice crystals will grow, or recrystallize to large ice crystals, which may cause damage to the food texture. Damage such as the physical rupture of cell walls and membranes and separation of plant and animal cells cause limp celery or green beans, and drips in thawed berries and meat. Enlarged ice crystals also disrupt emulsions (butter and milk), frozen foams (ice-cream), and gels (frozen pudding and pie fillings), thus making these frozen products less homogenous, creamy, and smooth.

Freezer Burn. Another quality damage relating to ice recrystallization is the freezer-burn problem. Freezer-burn occurs when there is a headspace in the packaged food and the food is subjected to fluctuating storage temperature. When the temperature increases, ice at the warmer surface will sublime into the headspace. As the temperature of the freezer or surroundings cools down, the water vapor will recrystallize on the inner surface of the package instead of going back into the product. This leads to dehydration of the surface of the product. If the frozen product is not packaged, the freezer-burn problem is more common and more severe.

1.5.2.4 Types of Common Freezers with Different Cooling Media

1. *Cold air Freezers.*
   a. *Blast/belt freezers* are large insulated tunnels in which air as cold as $-40^{\circ}C$ is circulated to remove heat. The process is cheap, simple, and geared toward high-volume production. Rotating spiral tiers and multilayered belts are incorporated to move product through quickly and avoid "hot spots".
   b. *Fluidized bed freezers* are modified blast freezers in which cold air is passed at a high velocity through a bed of food, contained on a perforated conveyor belt. This produces a high freezing rate, but is restricted to particulate foods (peas, shrimp, and strawberries).
2. *Cold Surface Freezers.*
   a. *Plate freezers* work by increasing the amount of surface area that comes into direct contact with the product to be frozen. Typically, refrigerant runs in the coils that run through plates or drums on which products are laid out. Double-plated systems further increase the rate of heat transfer to obtain higher

quality. This system is suitable for flat and uniform products such as fish fillets, beef burgers, and dinner meals.

b. *Scraped-surface freezers* work by having a liquid or semisolid food (ice-cream) frozen onto the surface of the freezer vessel, and a rotor scrapes the frozen portion from the wall. Typically, ice-cream is only partially frozen in a scraped-surface freezer to about $-6^{\circ}C$, and the final freezing is completed in a hardening room ($-30^{\circ}C$).

3. *Cold Liquid Freezers.* Brine freezers using supersaturated solutions for maximum surface contact immerse the product in a liquid freezing agent, in particular for irregularly shaped product, such as crabs. Their main disadvantage is that products are subject to absorption of salt as well as bacteria if not properly packed.
4. *Cryogenic Freezer.* Liquid nitrogen or liquid carbon dioxide (which vaporize at $-178^{\circ}C$ and $-80^{\circ}C$, respectively), freeze product extremely quickly. This is only suitable for freezing premium products such as shrimp and crab legs because of the high cost of the nonrecoverable gas.

Tips for obtaining top-quality frozen product include the following:

- Start with high-quality product – freezing can maintain quality but not enhance it.
- Get the heat out quickly, by removing any nonedible parts from the food.
- Maintain the integrity of the frozen product – proper cutting and packaging avoids drips.
- Store the product at the coldest temperature economically possible, in a well-designed and maintained facility. Use proper inventory techniques to avoid deterioration.
- Avoid temperature fluctuation during storage and shipping.

1.5.3 Evaporation and Drying

During food processing, evaporation is used to achieve the following goals:

- Concentrate food by partial removal of water;
- Remove undesirable food volatiles;
- Recover desirable food volatiles.

Traditionally, evaporation is achieved via the following methods:

- Using solar energy to evaporate water from seawater to recover the salts left behind;
- Using a heated kettle or similar equipment to boil water from liquid or semisolid foods, for example, sugar syrup;
- Using the improved method of evaporating under a vacuum, where the term vacuum evaporator refers to a closed heated kettle or similar equipment connected to a vacuum pump. One principle to remember is that a major objective of vacuum evaporators is to remove water at temperatures low enough to avoid heat damage to the food.

There are, at present, many specialized pieces of equipment used for evaporating food products. However, overall, the above three methods are most common.

Drying differs from evaporating in that the former takes the food to nearly total dryness or the equivalence of 97% or 98% solids. The oldest method of drying food is to put the food under the hot sun. This practice probably started thousands of years ago. Although sun drying is still practiced, especially in many Third World countries, modern food drying has been modified to a nearly exact science. Drying has multiple objectives:

- To preserve the food from spoilage;
- To reduce the weight and bulk of the food;
- To make the food enjoy an availability and consumption pleasure similar to canned goods;
- To develop "new" or "novelty" items such as snacks.

Some well-known products prepared from drying include

- Dried milk powder,
- Instant coffee,
- Fish and shellfish,
- Jerky,
- Dried fruits, and
- Dried potato flakes.

The central equipment in drying food is the dryer. There are many types of dryer: spray dryers: drum dryers, roller dryers, and others, each of which can be used to meet specific needs.

1.5.4 Food Additives

One popular method of food preservation is the addition of chemicals, legally known as food additives in the United States. In January of 1992, the U.S. FDA and the International Food Information Council released a brochure presenting an overview of food additives. The information in this section has been derived from this document, with an update.

Perhaps the main functional objectives of the use of food additives are the following:

- What keeps bread mold-free and salad dressings from separating?
- What helps cake batters rise reliably during baking and keeps cured meats safe to eat?
- What improves the nutritional value of biscuits and pasta, and gives gingerbread its distinctive flavor?
- What gives margarine its pleasing yellow color and prevents salt from becoming lumpy in its shaker?
- What allows many foods to be available year-round, in great quantity, and with the best quality?

Food additives play a vital role in today's bountiful and nutritious food supply. They allow our growing urban population to enjoy a variety of safe, wholesome, and tasty foods, year-round. Also, they make possible an array of convenience foods without the inconvenience of daily shopping.

Although salt, baking soda, vanilla, and yeast are commonly used in foods today, many people tend to think of any additive added to foods as being a complex and sometimes

harmful chemical compound. All food additives are carefully regulated by federal authorities and various international organizations to ensure that foods are safe to eat and are accurately labeled. The purpose of the brochure from which this information is taken is to provide helpful background information about food additives, why they are used in foods, and how regulations govern their safe use in the food supply.

1.5.4.1 Why are Additives Used in Foods? Additives perform a variety of useful functions in foods that are often taken for granted. As most people no longer live on farms, additives help keep food wholesome and appealing while en route to markets sometimes thousands of miles away from where it is grown or manufactured. Additives also improve the nutritional value of certain foods and can make them more appealing by improving their taste, texture, consistency, or color.

Some additives could be eliminated if we were willing to grow our own food, harvest and grind it, spend many hours on cooking, or accept increased risks of food spoilage. However, most people today have come to rely on the many technological, aesthetic, and convenience benefits that additives provide in food.

Additives are used in foods for five main reasons.

- *To Maintain Product Consistency*. Emulsifiers give products a consistent texture and prevent them from separating. Stabilizers and thickeners give smooth uniform texture. Anticaking agents help substances such as salt to flow freely.
- *To Improve or Maintain Nutritional Value*. Vitamins and minerals are added to many common foods such as milk, flour, cereal, and margarine to make up for those likely to be lacking in a person's diet or lost in manufacturing. Such fortification and enrichment have helped reduce malnutrition in the U.S. population. All products containing added nutrients must be appropriately labeled.
- *To Maintain Palatability and Wholesomeness*. Preservatives retard product spoilage caused by mold, bacteria, fungi, yeast, or air. Bacterial contamination can cause foodborne illness, including the life-threatening botulism. Antioxidants are preservatives that prevent fats and oils in baked goods and other foods from becoming rancid or developing an off-flavor. They also prevent cut fresh fruits such as apples from turning brown when exposed to air.
- *To Provide Leavening or Control Acidity/Alkalinity*. Leavening agents, which release acids when heated, can react with baking soda to help cakes, biscuits, and other baked goods to rise during baking. Other additives help modify the acidity and alkalinity of foods for proper flavor, taste, and color.
- *To Enhance Flavor or Impart Desired Color*. Many spices and natural and synthetic flavors enhance the taste of foods. Colors, likewise, enhance the appearance of certain foods to meet consumer expectations. Examples of substances that perform each of these functions are provided in Table 1.4.

Many substances added to food may seem foreign when listed on the ingredient label, but are actually quite familiar. For example, ascorbic acid is another name for Vitamin C; alpha-tocopherol is another name for Vitamin E; and beta-carotene is a source of Vitamin A. Although there are no easy synonyms for all additives, it is helpful to remember that all food is made up of chemicals. Carbon, hydrogen, and other chemical elements provide the basic building blocks for everything in life.

TABLE 1.4 Common Uses of Food Additives in Food Categories.

Common Uses of Additives and Examples*	Foods Where Likely Used
Impart/Maintain Desired Consistency	
Alginates, lecithin, mono- and diglycerides, methyl-cellulose, carrageenan, glyceride, pectin, guar gum, sodium aluminosilicate	Baked goods, cake mixes, salad dressings, ice-cream, process cheese, coconut, table salt
Improve/Maintain Nutritive Value	
Vitamins A and D, thiamine, niacin, riboflavin, pyridoxine, folic acid, ascorbic acid, calcium carbonate, zinc oxide, iron	Flour, bread, biscuits, breakfast cereals, pasta, margarine, milk, iodized salt, gelatin desserts
Maintain Palatability and Wholesomeness	
Propionic acid and its salts, ascorbic acid, butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), benzoates, sodium nitrite, citric acid	Bread, cheese, crackers, frozen and dried fruit, margarine, lard, potato chips, cake mixes, meat
Produce Light Texture; Control Acidity/Alkalinity	
Yeast, sodium bicarbonate, citric acid, fumaric acid, phosphoric acid, lactic acid, tartrates	Cakes, cookies, quick breads, crackers, butter, chocolates, soft drinks
Enhance Flavor or Impart Desired Color	
Cloves, ginger, fructose, aspartame, saccharin, FD&C Red No.40, monosodium glutamate, caramel, annatto, limonene, turmeric	Spice cake, gingerbread, soft drinks, yogurt, soup, confections, baked goods, cheeses, jams, gum

*Includes generally recognized as safe (GRAS) and prior-sanctioned substances as well as food additives.

1.5.4.2 What is a Food Additive? In its broadest sense, a food additive is any substance added to food. Legally, the term refers to "any substance the intended use which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food." This definition includes any substance used in the production, processing, treatment, packaging, transportation, or storage of food.

Direct Additive. If a substance is added to a food for a specific purpose in that food, it is referred to as a direct additive. For example, the low-calorie sweetener aspartame, which is used in beverages, puddings, yogurt, chewing gum, and other foods, is considered a direct additive. Many direct additives are identified on the ingredient label of foods.

Indirect Food Additives. Indirect food additives are those that become part of the food in trace amounts due to its packaging, storage, or other handling. For instance, minute amounts of packaging substances may find their way into foods during storage. Food packaging manufacturers must prove to the FDA that all materials coming in contact with food are safe, before they are permitted for use in such a manner.

1.5.4.3 What is a Color Additive? A color additive is any dye, pigment, or substance that can impart color when added or applied to a food, drug, or cosmetic, or to the human body. Color additives may be used in foods, drugs, cosmetics, and certain medical devices such as contact lenses. Color additives are used in foods for many reasons, including to offset color loss due to storage or processing of foods and to correct natural variations in food color.

Colors permitted for use in foods are classified as certified or exempt from certification. Certified colors are man-made, with each batch being tested by the manufacturer and FDA to ensure that they meet strict specifications for purity. There are nine certified colors approved for use in the United States. One example is FD&C Yellow No.6, which is used in cereals, bakery goods, snack foods, and other foods.

Color additives that are exempt from certification include pigments derived from natural sources such as vegetables, minerals, or animals. For example, caramel color is produced commercially by heating sugar and other carbohydrates under strictly controlled conditions for use in sauces, gravies, soft drinks, baked goods, and other foods. Most colors exempt from certification must also meet certain legal criteria for specifications and purity.

1.5.4.4 How are Additives Regulated? Additives are not always byproducts of twentieth-century technology or modern know-how. Our ancestors used salt to preserve meats and fish, added herbs and spices to improve the flavor of foods, preserved fruit with sugar, and pickled cucumbers in a vinegar solution. Over the years, however, improvements have been made in increasing the efficiency and ensuring the safety of all additives. Today, food and color additives are more strictly regulated than at any other time in history. The basis of modern food law in the United States is the Federal Food, Drug, and Cosmetic (FD&C) Act of 1938, which gives the FDA authority over food and food ingredients and defines requirements for truthful labeling of ingredients.

The Food Additives Amendment to the FD&C Act, passed in 1958, requires FDA approval for the use of an additive prior to its inclusion in food. It also requires the manufacturer to prove an additive's safety for the ways it will be used. The Food Additives Amendment exempted two groups of substances from the food additive regulation process. All substances that the FDA or the USDA had determined were safe for use in specific food prior to the 1958 amendment were designated as *prior-sanctioned* substances. Examples of prior-sanctioned substances are sodium nitrite and potassium nitrite, used to preserve cured meats. However, at present, nitrites are called color-fixing agents for cured meats, and not preservatives, according to the FDA. The second category of substances excluded from the food additive regulation process are *generally recognized as safe* or GRAS substances. These substances are those whose use is generally recognized by experts as safe, based on their extensive history of use in food before 1958 or based on published scientific evidence. Salt, sugar, spices, vitamins, and monosodium glutamate are classified as GRAS substances, along with several hundred other substances. Manufacturers may also request FDA to review the use of a substance to determine if it is GRAS.

Since 1958, FDA and USDA have continued to monitor all prior-sanctioned and GRAS substances in light of new scientific information. If new evidence suggests that a GRAS or prior-sanctioned substance may be unsafe, federal authorities can prohibit its use or require further studies to determine its safety.

In 1960, Congress passed similar legislation governing color additives. The Color Additives Amendments to the FD&C Act require dyes used in foods, drugs, cosmetics, and certain medical devices to be approved by FDA prior to their marketing. In contrast

to food additives, colors in use before the legislation were allowed continued use only if they underwent further testing to confirm their safety. Of the original 200 provisionally listed color additives, 90 have been listed as safe and the remainder have either been removed from use by FDA or withdrawn by industry.

Both the Food Additives and Color Additives Amendments include a provision that prohibits the approval of an additive if it is found to cause cancer in humans or animals. This clause is often referred to as the Delaney Clause, named for its Congressional sponsor, Rep. James Delaney (D-N.Y.).

Regulations known as Good Manufacturing Practices (GMP) limit the amount of food and color additives used in foods. Manufacturers use only the amount of an additive necessary to achieve the desired effect.

1.5.4.5 How are Additives Approved for Use in Foods? To market a new food or color additive, a manufacturer must first petition to FDA for its approval. Approximately 100 new food and color additives petitions are submitted to FDA annually. Most of these petitions are for indirect additives such as packaging materials.

A food or color additive petition must provide convincing evidence that the proposed additive performs as it is intended. Animal studies using large doses of the additive for long periods are often necessary to show that the substance would not cause harmful effects at expected levels of human consumption. Studies of the additive in humans also may be submitted to FDA.

In deciding whether an additive should be approved, the agency considers the composition and properties of the substance, the amount likely to be consumed, its probable long-term effects, and various safety factors. Absolute safety of any substance can never be proven. Therefore, FDA must determine if the additive is safe under the proposed conditions of use, based on the best scientific knowledge available.

If an additive is approved, FDA issues regulations that may include the types of foods in which it can be used, the maximum amounts to be used, and how it should be identified on food labels. Additives proposed for use in meat and poultry products also must receive specific authorization by USDA. Federal officials then carefully monitor the extent of Americans' consumption of the new additive and results of any new research on its safety to assure its use continues to be within safe limits.

In addition, FDA operates an Adverse Reaction Monitoring System (ARMS) to help serve as an ongoing safety check of all additives. The system monitors and investigates all complaints by individuals or their physicians that are believed to be related to specific foods, food and color additives, or vitamin and mineral supplements. The ARMS computerized database helps officials decide whether reported adverse reactions represent a real public health hazard associated with food, so that appropriate action can be taken.

In summary, additives have been used for many years to preserve, flavor, blend, thicken, and color foods, and have played an important role in reducing serious nutritional deficiencies among Americans. Additives help assure the availability of wholesome, appetizing, and affordable foods that meet consumer demands from season to season. Today, food and color additives are more strictly regulated than at any time in history. Federal regulations require evidence that each substance is safe at its intended levels of use before it may be added to foods. All additives are subject to an ongoing safety review as scientific understanding and methods of testing continue to improve.

1.5.4.6 Additional Information About Additives. Table 1.5 provides additional information about food additives.

TABLE 1.5 Answers to Some of the Most Popular Questions About Food Additives.

Q	*What is the difference between "natural" and "artificial" additives?*
A	Some additives are manufactured from natural sources such as soybeans and corn, which provide lecithin to maintain product consistency, or beets, which provide beet powder used as food coloring. Other useful additives are not found in nature and must be man-made. Artificial additives can be produced more economically, with greater purity and more consistent quality than some of their natural counterparts. Whether an additive is natural or artificial has no bearing on its safety.
Q	*Is a natural additive safer because it is chemical-free?*
A	No. All foods, whether picked from your garden or your supermarket shelf, are made up of chemicals. For example, the vitamin C or ascorbic acid found in an orange is identical to that produced in a laboratory. Indeed, all things in the world consist of the chemical building blocks of carbon, hydrogen, nitrogen, oxygen, and other elements. These elements are combined in various ways to produce the starches, proteins, fats, water, and vitamins found in foods.
Q	*Are sulfites safe?*
A	Sulfites added to baked goods, condiments, snack foods, and other products are safe for most people. A small segment of the population, however, has been found to develop hives, nausea, diarrhea, shortness of breath, or even fatal shock after consuming sulfites. For that reason, in 1986 FDA banned the use of sulfites on fresh fruits and vegetables intended to be sold or served raw to consumers. Sulfites added as a preservative in all other packaged and processed foods must be listed on the product label.
Q	*Does FD&C Yellow No. 5 cause allergic reactions?*
A	FD&C Yellow No. 5, or tartrazine, is used to color beverages, dessert powders, candy ice-cream, custards, and other foods. The color additive may cause hives in fewer than one out of 10,000 people. By law, whenever the color is added to foods or taken internally, it must be listed on the label. This allows the small portion of people who may be sensitive to FD&C Yellow No. 5 to avoid it. Actually, any certified color added to food is required to be listed on the label.
Q	*Does the low-calorie sweetener aspartame carry adverse reactions?*
A	There is no scientific evidence that aspartame causes adverse reactions in people. All consumer complaints related to the sweetener aspartame have been investigated as thoroughly as possible by federal authorities for more than five years, in part under FDA's Adverse Reaction Monitoring System. In addition, scientific studies conducted during aspartame's preapproval phase failed to show that it causes any adverse reactions in adults or children. Individuals who have concerns about possible adverse reactions to aspartame or other substances should contact their physicians.
Q	*Do additives cause childhood hyperactivity?*
A	No. Although this theory was popularized in the 1970s, well-controlled studies conducted since that time have produced no evidence that food additives cause hyperactivity or learning disabilities in children. A Consensus Development Panel of the National Institutes of Health concluded in 1982 that there was no scientific evidence to support the claim that additives or colorings cause hyperactivity.
Q	*Why are decisions sometimes changed about the safety of food ingredients?*
A	As absolute safety of any substance can never be proven, decisions about the safety of food ingredients are made on the best scientific evidence available. Scientific knowledge is constantly evolving. Therefore, federal officials often review earlier decisions to ensure that the safety assessment of a food substance remains up to date. Any change made in previous clearances should be recognized as an assurance that the latest and best scientific knowledge is being applied to enhance the safety of the food supply.

(*Continued*)

TABLE 1.5 ***Continued.***

Q	*What are some other food additives that may be used in the future?*
A	Among other petitions, FDA is carefully evaluating requests to use ingredients that would replace either sugar or fat in food. In 1990, FDA confirmed the GRAS status of Simplesse (registered trademark), a fat replacement made from milk or egg-white protein, for use in frozen desserts. The agency has also confirmed the use of the food additive Olestra, which will partially replace the fat in oils and shortenings.
Q	*What is the role of modern technology in producing food additives?*
A	Many new techniques are being researched that will allow the production of additives in ways not previously possible. One approach, known as biotechnology, uses simple organisms to produce additives that are the same food components found in nature. In 1990, FDA approved the first bioengineered enzyme, rennin, which traditionally has been extracted from calves' stomachs, for use in making cheese.

1.5.5 Fermentation

The availability of fermented foods has a long history among different cultures. Acceptability of fermented foods also differs among cultural habits. A product highly acceptable in one culture may not be so acceptable by consumers in another culture. The number of fermented food products is countless. Manufacturing processes of fermented products vary considerably due to variables such as food groups, form, and characteristics of final products, kinds of ingredients used, and cultural diversity. Fermented foods can be prepared from various products derived from dairies, grains, legumes, fruits, vegetables, muscle foods, and so on.

1.5.6 New Technologies

At present, some alternative or new technologies in food processing are available. On June 2, 2000, the U.S. FDA released a report titled "Kinetics of Microbial Inactivation for Alternative Food Processing Technologies." This report evaluates the scientific information available on a variety of alternative food-processing technologies. The purpose of the report is to help FDA evaluate each technology's effectiveness in reducing and inactivating pathogens of public health concern.

The information in this section has been completely derived from this report. For ease of reading, all references have been removed. Consult the original documents for unabridged data. The citation data for this document are: A report of the Institute of Food Technologists for the Food and Drug Administration of the U.S. Department of Health and Human Services, submitted March 29, 2000, revised June 2, 2000, IFT/FDA Contract No. 223-98-2333, Task Order 1, How to Quantify the Destruction Kinetics of Alternative Processing Technologies, http://www.cfsan.fda.gov/~comm/ift-pref.html.

This section will discuss briefly the following new technologies:

- Microwave and radio frequency processing,
- Ohmic and inductive heating,
- High-pressure processing,
- Pulse electric fields,
- High-voltage arc discharge,

- Pulse light technology,
- Oscillating magnetic fields,
- Ultraviolet light,
- Ultrasound, and
- Pulse X-rays.

1.5.6.1 Microwave and Radio Frequency Processing. Microwave and radio frequency heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material through two mechanisms, dielectric and ionic. Microwave and radio frequency heating for pasteurization and sterilization are preferred to conventional heating because they require less time to come up to the desired process temperature, particularly for solid and semisolid foods. Industrial microwave pasteurization and sterilization systems have been reported on and off for over 30 years, but commercial radio frequency heating systems for the purpose of food pasteurization or sterilization are not known to be in use.

For a microwave sterilization process, unlike conventional heating, the design of the equipment can dramatically influence the critical process parameters – the location and temperature of the coldest point. This uncertainty makes it more difficult to make general conclusions about processes, process deviations, and how to handle deviations. Many techniques have been tried to improve the uniformity of heating. The critical process factor when combining conventional heating and microwave or any other novel processes would most likely remain the temperature of the food at the cold point, primarily due to the complexity of the energy absorption and heat transfer processes. As the thermal effect is presumably the sole lethal mechanism, time–temperature history at the coldest location will determine the safety of the process and is a function of the composition, shape, and size of the food, the microwave frequency, and the applicator (oven) design. Time is also a factor in the sense that, as the food heats up, its microwave absorption properties can change significantly and the location of cold points can shift.

1.5.6.2 Ohmic and Inductive Heating. Ohmic heating (sometimes also referred to as Joule heating, electrical resistance heating, direct electrical resistance heating, electroheating, and electroconductive heating) is defined as the process of passing electric currents through foods or other materials to heat them. Ohmic heating is distinguished from other electrical heating methods either by the presence of electrodes contacting the food, frequency, or waveform.

Inductive heating is a process in which electric currents are induced within the food due to oscillating electromagnetic fields generated by electric coils. No data about microbial death kinetics under inductive heating have been published.

A large number of potential future applications exist for ohmic heating, including its use in blanching, evaporation, dehydration, fermentation, and extraction. The principal advantage claimed for ohmic heating is its ability to heat materials rapidly and uniformly, including products containing particulates. The principal mechanisms of microbial inactivation in ohmic heating are thermal. Although some evidence exists for nonthermal effects of ohmic heating, for most ohmic processes, which rely on heat, it may be unnecessary for processors to claim this effect in their process filings.

1.5.6.3 High-Pressure Processing (HPP). High-pressure processing (HPP), also described as high-hydrostatic-pressure (HHP) or ultra-high-pressure (UHP) processing, subjects liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa. Process temperature during pressure treatment can be specified from below 0°C to above 100°C. Commercial exposure times can range from a millisecond pulse to over 20 min. Chemical changes in the food generally will be a function of the process temperature and treatment time.

The HPP process acts instantaneously and uniformly throughout a mass of food independent of size, shape, and food composition. Compression will uniformly increase the temperature of foods approximately 3°C per 100 MPa. The temperature of a homogeneous food will increase uniformly due to compression. Compression of foods may shift the pH of the food as a function of imposed pressure and must be determined for each food treatment process. Water activity and pH are critical process factors in the inactivation of microbes by HPP. An increase in food temperature above room temperature and to a lesser extent a decrease below room temperature increases the inactivation rate of microorganisms during HPP treatment. Temperatures in the range of 45 to 50°C appear to increase the rate of inactivation of food pathogens and spoilage microbes. Temperatures ranging from 90 to 110°C in conjunction with pressures of 500–700 MPa have been used to inactivate spore-forming bacteria such as *Clostridium botulinum*. Current pressure processes include batch and semicontinuous systems, but no commercial continuous HPP systems are operating.

The critical process factors in HPP include pressure, time at pressure, time to achieve treatment pressure, decompression time, treatment temperature (including adiabatic heating), product initial temperature, vessel temperature distribution at pressure, product pH, product composition, product water activity, packaging material integrity, and concurrent processing aids. Other processing factors present in the process line before or after the pressure treatment are not included.

Because some types of spores of *C. botulinum* are capable of surviving even the most extreme pressures and temperatures of HPP, there is no absolute microbial indicator for sterility by HPP. For vegetative bacteria, nonpathogenic *L. innocua* is a useful surrogate for the foodborne pathogen *L. monocytogenes*. A nonpathogenic strain of *Bacillus* may be useful as a surrogate for HPP-resistant *E. coli* O157:H7 isolates.

1.5.6.4 Pulsed Electric Fields (PEF). High-intensity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20–80 kV/cm) to foods placed between two electrodes. The process may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses and at ambient, subambient, or slightly above ambient temperatures for less than 1 s. Energy loss due to heating of foods is minimized, reducing detrimental changes in the sensory and physical properties of foods.

Some important aspects in PEF technology are the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods with minimum increase in temperature, and the design of electrodes that minimize the effect of electrolysis. Although different laboratory- and pilot-scale treatment chambers have been designed and used for PEF treatment of foods, only two industrial-scale PEF systems are available. The systems (including treatment chambers and power supply equipment) need to be scaled up to commercial systems.

To date, PEF has been applied mainly to improve the quality of foods. Application of PEF is restricted to food products that can withstand high electric fields, have low electrical conductivity, and do not contain or form bubbles. The particle size of the liquid food in both static and flow treatment modes is a limitation.

Several theories have been proposed to explain microbial inactivation by PEF. The most studied are electrical breakdown and electroporation. Factors that affect microbial inactivation with PEF are process factors (electric field intensity, pulse width, treatment time and temperature, and pulse wave shapes), microbial entity factors (type, concentration, and growth stage of the microorganism), and media factors (pH, antimicrobials, and ionic compounds, conductivity, and medium ionic strength).

Although PEF has potential as a technology for food preservation, existing PEF systems and experimental conditions are diverse, and conclusions about the effects of critical process factors on pathogens of concern and kinetics of inactivation need to be further studied.

1.5.6.5 High-Voltage Arc Discharge. Arc discharge was an early application of electricity for pasteurization of fluids by applying rapid discharge voltages through an electrode gap below the surface of an aqueous suspension of microorganisms. A multitude of physical effects (intense wave) and chemical compounds (electrolysis) are generated, inactivating the microorganisms. The use of arc discharge for liquid foods may be unsuitable, largely because electrolysis and the formation of highly reactive chemicals occur during the discharge. More recent designs may show some promise for use in food preservation, although the reported results should be confirmed by independent researchers.

1.5.6.6 Pulsed Light Technology. Pulsed light is a method of food preservation that involves the use of intense and short-duration pulses of broad spectrum "white light" (ultraviolet to the near infrared region). For most applications, a few flashes applied in a fraction of a second provide a high level of microbial inactivation. This technology is applicable mainly in sterilizing or reducing the microbial population on packaging or food surfaces. Extensive independent research on the inactivation kinetics under a full spectrum of representative variables of food systems and surfaces is needed.

1.5.6.7 Oscillating Magnetic Fields. Static (SMF) and oscillating (OMF) magnetic fields have been explored for their potential to inactivate microorganisms. For SMFs, the magnetic field intensity is constant with time, but an OMF is applied in the form of constant amplitude or decaying amplitude sinusoidal waves. An OMF applied in the form of pulses reverses the charge for each pulse. The intensity of each pulse decreases with time to about 10% of the initial intensity. Preservation of foods with OMF involves sealing food in a plastic bag and subjecting it to 1 to 100 pulses in an OMF with a frequency between 5 and 500 kHz at temperatures of 0 to 50°C for a total exposure time ranging from 25 ms to 100 ms.

The effects of magnetic fields on microbial populations have produced controversial results. Consistent results concerning the efficacy of this method are needed before considering this technology for food preservation purposes.

1.5.6.8 Ultraviolet Light. There is a particular interest in using ultraviolet (UV) light to treat fruit juices, in particular apple juice and cider. Other applications include disinfection of water supplies and food contact surfaces. Ultraviolet processing involves the use of radiation from the UV region of the electromagnetic spectrum. The germicidal properties

of UV irradiation (UVC 200–280 nm) are due to DNA mutations induced by DNA absorption of the UV light. This mechanism of inactivation results in a sigmoidal curve of microbial population reduction.

To achieve microbial inactivation, the UV radiant exposure must be at least 400 J/m^2 in all parts of the product. Critical factors include the transmissivity of the product, the geometric configuration of the reactor, the power, wavelength, and physical arrangement of the UV source(s), the product flow profile and the radiation path length. Ultraviolet light may be used in combination with other alternative process technologies, including various powerful oxidizing agents such as ozone and hydrogen peroxide, among others.

1.5.6.9 Ultrasound. Ultrasound is energy generated by sound waves of 20,000 or more vibrations per second. Although ultrasound technology has a wide range of current and future applications in the food industry, including inactivation of microorganisms and enzymes, most current developments for food applications are nonmicrobial.

Data on inactivation of food microorganisms by ultrasound in the food industry are scarce, and most applications use it in combination with other preservation methods. The bactericidal effect of ultrasound is attributed to intracellular cavitations, that is, micromechanical shocks that disrupt cellular structural and functional components up to the point of cell lysis. The heterogeneous and protective nature of food with the inclusion of particulates and other interfering substances severely curtails the singular use of ultrasound as a preservation method. Although these limitations make the current probability of commercial development low, combination of ultrasound with other preservation processes (e.g., heat and mild pressure) appears to have the greatest potential for industrial application.

Critical processing factors are assumed to be the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, the type of microorganism, the volume of food to be processed, the composition of the food, and the temperature of treatment.

1.5.6.10 Pulsed X-Rays. It is important to realize that pulsed X-ray is one form of irradiation that has been applied to the preservation of several categories of food in the United States. Electrons have a limited penetration depth of about 5 cm in food, but X-rays have significantly higher penetration depths (60–400 cm), depending upon the energy used.

Pulsed X-ray is a new alternative technology that utilizes a solid state-opening switch to generate electron beam X-ray pulses of high intensity (opening times from 30 ns down to a few nanoseconds; repetition rates up to 1000 pulses/s in burst mode operation). The specific effect of pulses in contrast to nonpulsed X-rays has yet to be investigated.

The practical application of food irradiation by X-rays in conjunction with existing food processing equipment is further facilitated by (1) the possibility of controlling the direction of the electrically produced radiation; (2) the possibility of shaping the geometry of the radiation field to accommodate different package sizes; and (3) its high reproducibility and versatility.

Potentially, the negative effects of irradiation on food quality can be reduced.

1.6 PACKAGING

The obvious reason for packaging a food product is to protect the food so its elements will not be exposed until it is ready to be prepared and consumed. In the world of food

manufacturing, this is not a small matter, because the FDA has rigid control over the materials used in food packaging. As far as the FDA is concerned, any packaging material is considered a food additive. All packaging materials used to contain food must comply with rigid regulations for the use of a food additive. The term "food additive" means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; if such substance is not generally recognized as safe). Recently, the FDA has established the Food Contact Notification Program. It issues administrative guidance and regulations for the use of packaging materials, among others. FDA's website (www.FDA.gov) provides details for this program.

Different materials are used as packaging containers, including, but not limited to

- Glass,
- Plastic,
- Laminates (paper-based), and
- Metal can.

Most chapters in this book contain a section on food packaging.

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2

Fermented Products and Their Manufacture

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2.1 Introduction 46
2.2 Fermented Dairy Products 46
2.2.1 Ingredients and Kinds of Products 46
2.2.2 Cheeses 48
2.2.2.1 Cottage Cheese Manufacturing 54
2.2.2.2 Cheddar Cheese Manufacturing 54
2.2.2.3 Swiss Cheese Manufacturing 55
2.2.2.4 Blue Cheese 55
2.2.2.5 American-Style Camembert Cheese 56
2.2.2.6 Feta Cheese Manufacturing 57
2.2.3 Yogurt 57
2.2.4 Fermented Liquid Milks 59
2.2.4.1 Sour Milk Manufacturing 60
2.2.4.2 Kefir Manufacturing 62
2.2.4.3 Acidophilus Milk 63
2.3 Meat Products 63
2.3.1 Ingredients and Types 63
2.3.2 Hams 63
2.3.3 Sausages 65
2.4 Fermented Cereal Products (Breads and Related Products) 66

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

2.4.1 Types of Products and Ingredients 66
2.4.2 Regular Bread 66
2.4.3 Retarded Dough 69
2.4.4 Flat (Layered) Bread 69
2.4.5 Croissants and Danish Pastries 70
2.4.6 Steamed Bread (Mantou) 70
2.5 Fermented Soy Products 71
2.5.1 Types of Products and Ingredients 71
2.5.2 Soy Sauce 73
2.5.3 Fermented Whole Soybeans 73
2.5.3.1 Ordinary (Itohiki) Natto 73
2.5.3.2 Hama-natto and Douchi 73
2.5.4 Fermented Soy Pastes 74
2.5.5 Fermented Tofu 75
2.5.5.1 Sufu or Fermented Soy Cheese 75
2.5.5.2 Stinky Tofu 75
2.5.6 Tempe (Tempeh) 76
2.6 Fermented Vegetables 77
2.6.1 Types of Products and Ingredients 77
2.6.2 Sauerkraut 78
2.6.3 Pickles 78
2.6.4 Kimchi 79
2.6.5 Chinese Pickled Vegetables 79
2.7 Application of Biotechnology in the Manufacturing of Fermented Foods 80
2.8 Process Mechanization in the Manufacture of Fermented Foods 80
References 81

2.1 INTRODUCTION

The availability of fermented foods has a long history in many different cultures. The acceptability of fermented foods also differs because of cultural habits. A product highly acceptable in one culture may not be so acceptable to consumers in another culture. There are countless fermented food products. The manufacturing processes for these fermented products vary considerably owing to variables such as food groups, form and characteristics of the final products, kind of ingredients used, and cultural diversity. It is beyond the scope of this chapter to address all the manufacturing processes used to produce fermented foods. Instead, this chapter is organized based on food groups such as dairy, meat, cereal, soy, and vegetables. Within each food group, manufacturing processes of typical products are examined. This chapter is only an introduction to manufacturing processes for selected fermented food products. Readers should consult the references at the end of the chapter, and other available literature, for detailed information.

2.2 FERMENTED DAIRY PRODUCTS

2.2.1 Ingredients and Kinds of Products

Fermented dairy products are commonly made in milk-producing countries and by nomadic people. These products are highly acceptable in these cultures. They have been gradually accepted by other cultures as a result of cultural exchange. It is generally accepted that

most fermented dairy products were first discovered and developed by nomadic people. The production of a fermented dairy product nowadays can be a highly sophisticated process. However, it can still be conducted in a fairly primitive manner in another location. The quality of a fermented dairy product varies due to the milk, microorganisms, and other ingredients used in the manufacturing process. Many factors affect the gross composition of milk (Jenness 1988; Kosikowski and Mistry 1997; Early 1998; Spreer, 1998; Walstra and others 1999). The factors most significant to the processing of milk products are breed, feed, season, region, and herd health. Reviews of animals' milk are available in the literature. Table 2.1 lists the approximate composition of cow's milk (Robinson 1986; Jenness 1988; Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Walstra and others 1999). In industrial countries, milk composition is standardized to meet a country's requirements. However, it is understood that the requirements in one country may not be the same as those in another; thus, the composition may vary for the same product. International agreements to standardize some products are now available. However, products made in different locations can still vary because of microorganisms and culturing practices used in their production.

Fermented dairy products can be grossly divided into three main categories: cheeses, yogurts, and fermented liquid milks. Within each of these categories, there are subcategories. Table 2.2 presents examples for each of these categories (Robinson 1986; Jenness 1988; Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Walstra and others 1999).

In the manufacturing of fermented dairy products, various ingredients, such as the milk itself, microorganism(s), coagulants, salt, sugar, vitamins, buffering salts, bleaching (decolorizing) agents, dyes (coloring agents), flavoring compounds, stabilizers and emulsifiers, may be used. The uses of these ingredients in fermented liquid milks, yogurts, and natural and processed cheeses are summarized in Table 2.3 (Robinson 1986; Jenness 1988; Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Walstra and others 1999).

Various microorganisms, such as lactic acid bacteria, yeasts, and molds, are used in the manufacturing of fermented dairy products to give the various characteristics of these products. Table 2.4 lists some of the more common dairy microorganisms and their uses in fermented liquid dairy products, yogurts, and cheeses (Davies and Law 1984; Robinson 1990; Jay 1996). Cultures of the different microorganisms are available in various forms, such as liquid, frozen, or freeze-dried. Examples of their usage in the manufacturing of fermented dairy products are listed in Table 2.5 (Robinson 1986; Jenness 1988;

TABLE 2.1 Approximate Composition of Cow's Milk.

Components	Average Content in Milk (% w/w)	Range (% w/w)	Average Content in Dry Matter (% w/w)
Water	87.1	85.3–88.7	
Solid-not-fat	8.9	7.9–10.0	–69
Fat in dry matter	31	22–38	–31
Lactose	4.6	3.8–5.3	36
Fat	4	2.5–5.5	31
Protein	3.25	2.3–4.4	25
Casein	2.6	1.7–3.5	20
Mineral substances	0.7	0.57–0.83	5.4
Organic acids	0.17	0.12–0.21	1.3
Miscellaneous	0.15		1.2

Source: Robinson (1986), Jenness (1988), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

TABLE 2.2 Types of Fermented Dairy Products with Examples.

Kinds	Examples
Fermented Liquid Milks	
Lactic fermentation	Buttermilk, Acidophilus,
With alcohol and lactic acid	Kefir, Koumiss
With mold and lactic acid	Villi
Concentrated	Ymer, Skyr, Chakka
Yogurts	
Viscous/liquid	Yogurt
Semi-solid	Strained yogurt
Solid	Soft/hard frozen yogurt
Powder	Dried yogurt
Cheeses	
Extra hard	Parmesan, Romano, Sbrinz
Hard with eyes	Emmental, Gruyere, Swiss
Hard without eyes	Cheddar, Chester, Provolone
Semi-hard	Gouda, Edam, Caerphilly
Semi-hard, internally mold ripened	Roquefort, Blue, Gorgonzola
Semi-soft, surface-ripened with bacteria	Limburger, Brick, Munster
Soft, surface mold ripened	Brie, Camembert, Neufchatel
Soft, unripened	Cream, Mozzaella, U.S. cottage

Source: Robinson (1986), Jenness (1988), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Walstra and others 1999). Because the starter cultures are available in different forms, the preparation steps for these cultures before inoculation are different. Table 2.6 lists some of the preparation procedures used in the industry for different forms of starter cultures (Robinson 1986; Jenness 1988; Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Walstra and others 1999). Different microorganisms have different temperature requirements for their optimum growth and functioning. Some fermented dairy products, such as mold-ripened cheeses, may require more than one microorganism to complete the manufacturing process. These molds function best during the long ripening period and therefore have standard incubation temperatures in the refrigerated range. This is also true for some cheeses that require long ripening periods. Microorganisms requiring higher incubation temperatures are used in the production of fermented liquid milks that require only a short incubation time. Table 2.7 lists some of the dairy microorganisms used and their incubation temperatures (Davies and Law 1984; Robinson 1990; Nath 1993; Jay 1996; Law 1997; Specialist Cheese Association 1997; Scott and others 1998; Emmons 2000).

2.2.2 Cheeses

Cheeses can be classified into different categories based on their moisture, the way the milk is processed, and the types of microorganisms used for the ripening process (Table 2.8) (Robinson 1986; Jenness 1988; Early 1998). In the processing of cheese, the amount of curd used for each block of cheese differs considerably, and thus produces different block weights (Table 2.9) (Robinson 1986; Jenness 1988; Nath 1993;

TABLE 2.3 Ingredients for Fermented Dairy Food Production.

Ingredients	Fermented Liquid Milk Products	Yogurt	Natural Cheese	Processed Cheese Products
		Milk		
Raw	Optional	Optional	Optional	Optional
Standardized (fat and milk solids)	Preferred	Preferred	Preferred	Preferred
Milk powders	Optional	Optional	Optional	Optional
		Microorganisms		
Starter bacteria	Required	Required	Required	Required
Mold	Optional	Optional	Optional	Optional
Yeast	Optional	Optional	Optional	Optional
Genetically modified microorganisms	Optional	Optional	Optional	Optional
		Coagulant		
Rennet	Preferred	Preferred	Preferred	Preferred
Acid	Optional	Optional	Optional	Optional
Microbial protease(s)	Optional	Optional	Optional	Optional
Common salt (sodium chloride)	No	No	Required	Required
Sugar	Optional	Optional	No	No
Vitamins	Preferred	Preferred	Preferred	Preferred
Buffering salts (calcium chloride hydroxide phosphates, sodium or potassium phosphates)	Optional	Optional	Optional	Optional
Bleaching (decolorizing) agents	No	No	Optional	Optional
Antimicrobial agents	Optional	Optional	No	Preferred
Dyes (coloring agents)	No	No	Optional	Optional
Flavoring compounds (fruits, spices spice oils, fruits, fruit flavors, artificial smoke)	Optional	Optional	Optional	Optional
Stabilizers	No	Preferred	No	Preferred
Emulsifiers	Optional	Optional	No	Preferred

Source: Robinson (1986), Jenness (1988), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

Kosikowski and Mistry 1997; Law 1997; Specialist Cheesemakers Association 1997; Early 1998; Scott and others 1998). Harder cheeses are produced in much larger blocks than the soft cheeses. This may be due to their ease of handling after ripening.

Cheeses are packaged in different forms to satisfy consumer consumption patterns and to some extent to be compatible with the way the cheese is ripened and for marketing purposes. The various packaging materials are selected to protect the cheeses in a sanitary condition, extend shelf-life and delay the deterioration of the final product. Table 2.10 lists some of the requirements of cheese packaging materials (Robinson 1986; Kosikowski and Mistry 1997; Nath 1993; Specialist Cheesemakers Association 1997; Early 1998; Spreer 1998; Scott and others 1998; Walstra and others 1999; Emmons 2000).

TABLE 2.4 Some Common Organisms Used in Fermented Milk Products.

Microorganisms	Buttermilk	Cream	Fermented Milk	Yogurt	Kefir	Cheese
Bifidobacterium bifidum			X		X	X
Enterococcus durans						X
Enterococcus faecalis						X
Geotrichum candidum						X
Lactobacillus acidophilus			X			
Lactobacillus casei						X
Lactobacillus delbrueckii subsp. bulgaricus	X	X				X
Lactobacillus heleveticus						X
Lactobacillus kefir					X	
Lactobacillus lactis						X
Lactobacillus lactis biovar.diacetylactis		X				X
Lactobacillus lactis subsp. cremoris	X	X				X
Lactobacillus lactis subsp. lactis						X
Lactobacillus lactis var. hollandicus						X
Leuconostoc mesenteroidis subsp. cremoris						X
Leuconostoc mesenteroides subsp. dextranicum						X
Propionibacterium freudenreichii subsp. shermanii						X
Penicillium camemberti						X
Penicillium glaucum						X
Penicillium roqueforti						X
Streptococcus thermophilus				X		X

Source: Davies and Law (1984), Robinson (1990), Jay (1996).

All cheeses produced must be coagulated from acceptable milk to form the curd, followed by removal of the whey. Most cheeses are made from standardized and pasteurized milk. Nonpasteurized milk is also used in some exceptional cases, provided that it does not carry pathogens. The majority of cheeses are made from cow's milk. Milks from other animals are also used for specialty products. The coagulation process is conducted through the addition of a coagulant (rennin or chymosin) and incubation of appropriate lactic acid bacteria in milk to produce enough acid and appropriate pH for curdling of the milk. After the casein is recovered, it is salted and subjected to fermentation, with or without inoculation with other microorganisms, to produce the desirable characteristics of the various cheeses. Variations in the different manufacturing steps thus produce a wide variety of cheeses with different characteristics. Table 2.11 summarizes the basic steps in the cheese manufacturing process (Davies and Law 1984; Jenness 1988; Robinson 1986; Robinson 1990; Nath 1993; Jay 1996; Kosikowski and Mistry 1997; Specialist Cheesemakers Association 1997; Early 1998; Scott and others 1998; Spreer 1998;

TABLE 2.5 Dairy Starter Cultures.

Physical Form	Usage
Liquid cultures in skim milk or whole milk (antibiotic free)	For inoculation of intermediate cultures
Liquid culture – frozen	For inoculation of intermediate cultures For inoculation into bulk cultures
Dried culture – from normal liquid culture	For inoculation of intermediate cultures
Spray-dried cultures	For inoculation into bulk cultures For direct-to-vat inoculation.
Frozen cultures in special media (frozen at −40°C)	For inoculation into bulk cultures For direct-to-vat inoculation
Frozen concentrated culture (in sealed containers at −196°C)	For inoculation into bulk cultures For direct-to-vat inoculation
Single-strain lypholized cultures (in foil sachets with known activity)	For inoculation into bulk cultures For direct-to-vat inoculation

Source: Robinson (1986), Jenness (1988), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

Walstra and others 1999). Table 2.12 summarizes the ripening conditions for various cheeses. Selected examples are introduced in the following to provide an overview of the complexity of cheese manufacturing (Davies and Law 1984; Robinson 1986; 1990; Jenness 1988; Robinson and Tamime 1991; Nath 1993; Jay 1996; Kosikowski and

TABLE 2.6 Types of Starter Cultures and Their Preparation Prior to Usage.

Types of Culture	Preparation Steps	Timing
Regular starter culture	Preparation of starter culture blanks	8:00 A.M.
	Storing of milk blanks	11:00 A.M.
	Activating lypholized culture powder	3:00 P.M.
	Daily mother culture preparation	3:00 P.M.
	Semi-bulk and bulk starter preparation	3:00 P.M.
Frozen culture and bulk starter application	Store frozen culture at −40°C or less	
	Warm to 31°C and use directly	
Reconstituted milk or whey-based starter	Reconstitution	8:00 A.M.
	Heating and tempering	8:30 A.M.
	Inoculating and incubating	10:00 A.M.
Bulk starter from ultrafil milk	Ultrafiltration	1:00 P.M.
	Heating and tempering	3:30 P.M.
	Inoculating and incubating	5:00 P.M.

Source: Robinson (1986), Jenness (1988), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

TABLE 2.7 Temeprature Requirements and Acid Production for Some Dairy Microbes.

Microorganisms	Product Group	Standard Temperature for Incubation (°C)	General Maximun Titratable Acidity Produced in Milk (%)
	Bacteria		
Bifidobacterium bifidum	1	36–38	0.9–1.0
Lactobacillus acidophilus	1	38–44	1.2–2.0
Lactobacillus delbrueckii subsp. bulgaricus	1	43–47	2.0–4.0
Lactobacillus lactis subsp. cremoris	2	22	0.9–1.0
Lactobacillus subsp. lactis	2	22	0.9–1.0
Leuconostoc mesenteroides subsp. cremoris	2	20	0.1–0.3
Streptococcus durans	2	31	0.9–1.1
Streptococcus thermophilus	2	38–44	0.9–1.1
	Molds		
Penicillium roqueforti	3	11–16	NA
Penicillium camemberti	3	10–22	NA

Product group: 1, yogurt; 2, fermented liquid milk; 3, cheese.

Source: Davies and Law (1984), Robinson (1990), Nath (1993), Jay (1996), Law (1997), Specialist Cheesemakers Association (1997), Scott and others (1998), Emmons (2000).

TABLE 2.8 Classification of Cheese According to Moisture Content, Scald Temperature, and Method of Ripening.

Method	Cheese
Hard Cheese (*moisture 20–42%; fat-in-dry-matter 32–50%, min.*)	
Low scald, lactic starter	Gouda, Cheshire
Medium scald, lactic starter	Cheddar, Svecia
High scald, propionic eyes	Parmesan, Beaufort
Plastic curd, lactic starter or propionic eyes	Provolone Mozzarell
Semi-Hard Cheese (*moisture 43–55%; fat-in-dry-matter 40–50%, min.*)	
Lactic starter	St. Paul, Lanchester
Smear coat	Limburg, Munster
Blue veined mold	Roquefort Danablue
Soft Cheese (*moisture >55%; fat-in-dry-matter 4–51%, min.*)	
Acid-coagulated	Cottage cheese (U.S.), Quesco-Blanco
Smear coat or surface mold	Brie, Bel Paese
Surface mold	Camembert, Neufchatel
Normal lactic starter	Quarg, Petit Suisse
Unripened fresh	Cottage (U.K.), York

Source: Robinson (1986), Jenness (1988), Early (1998).

TABLE 2.9 Approximate Weight of Cheese Varieties.

Cheese Variety	Approximate Weight (kg)
Hard to Semi-Hard or Semi-Soft	
Wensleydale	3–5
Caerphilly	3–6
White Stilton	4–8
Single Gloucester	10–12
Leicester	13–18
Derby	14–16
Sage Derby	14–16
Cheddar	18–28
Cheshire	20–22
Dunlap	20–27
Double Gloucester	22–28
Lancashire	22
Internally Mold-Ripened (Blue-Veined) Cheese	
Blue Wensleydale	3–5
Blue Vinney	3–7
Blue Stilton	6–8
Blue Cheshire	10–20
Soft Cheese	
Colwich	0.25–0.50
Cambridge	0.25–1.0
Melbury	2.5

Source: Robinson (1986), Jenness (1988), Kosikowski and Mistry (1997), Law (1997), Specialist Cheesemakers Association (1997), Early (1998), Scott and others (1998).

TABLE 2.10 Requirements of Cheese Packaging Materials.

Low permeability to oxygen, carbon dioxide, and water vapor
Strength and thickness of film
Stability under cold or warm conditions
Stability to fats and lactic acid
Resistance to light, especially ultraviolet
Ease of application, stiffness, and elasticity
Ability to seal and accept adhesives
Laminated films to retain lamination
Low shrinkage or aging unless shrinkage is a requisite
Ability to take printed matter
Should not impart odors to the cheese
Suitability for mechanization of packaging
Hygienic considerations in storage and use
Cost-effectiveness as a protective wrapping

Source: Robinson (1986), Nath (1993), Kosikowski and Mistry (1997), Specialist Cheesemakers Association (1997), Early (1998), Scott and others (1998), Spreer (1998), Walstra and others (1999), Emmons (2000).

TABLE 2.11 Basic Cheese-Making Steps.

Standardization of cheese milks
Homogenization of cheese milks
Heat treatment or pasteurization of cheese milks
Starter addition
Addition of color and additives
Coagulation/curdling
Cutting the coagulum/curd
Stirring and scalding
Washing of curd cheese
Salting of cheese
Pressing of cheese
Coating, bandaging, and wrapping of cheese
Ripening
Retail packaging
Storage

Source: Davies and Law (1984), Robinson (1986), Jenness (1988), Nath (1993), Jay (1996), Kosikowski and Mistry (1997), Specialist Cheesemakers Association (1997), Early (1998), (1990), Scott and others (1998), Spreer (1998), Walstra and others (1999).

Mistry 1997; Specialist Cheesemakers Association, 1997; Early 1998; Spreer 1998; Scott and others 1998; Watsra and others 1999).

2.2.2.1 Cottage Cheese Manufacturing. Cottage cheese is a product that has had very mild fermentation treatment. It is produced by incubating (fermenting) the standardized and pasteurized skim milk with the starter lactic acid bacteria to produce enough acid and appropriate pH for the curdling of milk. The curd is then recovered and washed, followed by optional salting and creaming. The product is then packed and ready for marketing. No further ripening is required for this product. This is different from most fermented cheeses, which require a ripening process. Table 2.13 lists the various steps involved in the production of cottage cheese (Robinson 1986; Nath 1993; Kosikowski and Mistry 1997; Early 1998; Scott and others 1998; Spreer 1998; Walstra and others 1999).

2.2.2.2 Cheddar Cheese Manufacturing. Cheddar cheese is a common hard cheese without eyes that is used in the fast-food industry and in the household. Its production process is characterized by a requirement for milling and cheddaring of the curd. This cheese can be ripened with a wax rind or can be rindless (sealed under vacuum in plastic bags.) It is also

TABLE 2.12 Cheese Ripening Conditions.

Types of Cheese	Storage Period (days)	Temperature (°C)	Relative Humidity (%)
Soft	12–30	10–14	90–95
Mold-ripened	15–60	4–12	85–95
Cooked (e.g., Emmental)			
Cold room	7–25	10–15	80–85
Warm room	25–60	18–25	80–85
Hard (e.g., Cheddar)	45–360	5–12	87–95

Source: Davies and Law (1984), Robinson (1986, 1990), Jenness (1988), Robinson and Tamime (1991), Nath (1993), Jay (1996), Kosikowski and Mistry (1997), Specialist Cheesemakers Association (1997), Early (1998), Scott and others (1998), Spreer (1998), Walstra and others (1999).

TABLE 2.13 Basic Steps in Making Cottage Cheese.

Standardize the skim milk
Pasteurize the milk with standard procedure and cool to 32°C
Inoculate active lactic starter, add rennet, and set the curd
Rennet addition: at 2 ml single strength (prediluted, 1 : 40) per 1000 kg milk within 30 min of starter addition

Type of activity	Short set	Medium set	Long set
Starter concentration	5%	3%	0.5%
Temperature of milk set	32°C	27°C	22°C
Setting to cutting	5 h	8 h	14–16 h

Final pH and whey titratable acidity are 4.6 and 0.52%, respectively

Cut the curd with 1.3; 1.6; or 1.9-cm wire cheese knife
Cook the curd:
Let curd cubes stand for 15–30 min and cook to 51–54°C with 1.7°C per 10 min
Roll the curds gently every 10 min after initial 15–30 min wait
Test curd firmness and hold 10–30 min longer to obtain proper firmness
Wash the curd
First wash with 29°C water temperature
Second wash with 16°C water temperature
Third wash with 4°C water temperature
Gravitationally drain the washed curd for about 2.5 hr
Salt and cream at 152 kg creaming mixture per 454 kg with final 0.5–0.75% salt content and 4% fat content (varies with products and optional)
Package in containers
Store at refrigerated temperature

Source: Robinson (1986), Kosikowski and Mistry (1997), Early (1998), Scott and others (1998), Speer (1998), Walstra and others (1999).

categorized into regular, mild, or sharp based on the aging period (45–360 days). The longer the aging period, the sharper is the flavor, and it is sometimes more costly. It is packaged as a large block or in slices. Table 2.14 lists the basic steps in the manufacture of cheddar cheese (Robinson 1986; Nath 1993; Kosikowski and Mistry 1997; Early 1998; Scott and others 1998; Spreer 1998; Walstra and others 1999).

2.2.2.3 Swiss Cheese Manufacturing. Swiss cheese is also a common cheese used in the fast-food industry and in the household. It is characterized by having irregular eyes inside the cheese. These eyes are produced by *Propionicbacterium freudenreichii* subsp. shermanii, which generates gases that are trapped inside the block of cheese during fermentation and ripening. A cheese with eyes like Swiss cheese has become an icon for cheese in graphics. Swiss cheese is also characterized by its propionic acid odor. The salting process for Swiss cheese utilizes both the dry- and brine-salting processes. Like cheddar cheese, it can be categorized into regular, mild, and sharp depending on the length of the curing process. Table 2.15 lists the basic steps in the manufacture of Swiss cheese (Robinson 1986; Nath 1993; Kosikowski and Mistry 1997; Early 1998; Scott and others 1998; Spreer 1998; Walstra and others 1999).

2.2.2.4 Blue Cheese. Blue cheese is characterized by its strong flavor and by blue mold filaments generated by *Penicillium roqueforti* inside the cheese. It is commonly

TABLE 2.14 Basic Steps in the Making of Cheddar Cheese.

Standardize the cheese milk
Homogenize the milk
Pasteurize and additionally heat the milk
Cool the milk to 31°C
Inoculate the milk with lactic starter (0.5–2% active mesophilic lactic starter)
Add rennet or other protease(s): 198 ml single strength (1 : 15,000) rennet per 1000 kg milk. Dilute the measured rennet 1 : 40 before use. Agitate at medium speed.
Set the milk to the proper acidity, 25 min
Cut the curd using 0.64-cm or wider wire knife. Stir for 5 min at slow speed
Cook the curd at 38°C for 30 min with 1°C every 5 min increment. Maintain temperature for another 4–5 min and agitate periodically at medium speed.
Drain the curd at 38°C
Cheddar the curd at pH 5.2–5.3
Mill the curd slabs
Salt the curd at 2.3–3.5 kg salt per 100 kg curd in 3 portions in 30 min
Waxed cheddar cheese
Hoop and press at 172 kPa for 30–60 s then 172–344 kPa overnight
Dry the cheese at 13°C at 70% relative humidity for 2–3 days
Paraffin whole cheese at 118°C for 6 sec
Rindless cheddar cheese
Press at 276 kPa for 6–18 h
Pre-press for 1 min followed by 45 min under 686 mm vacuum
Remove and press at 345 kPa for 60 min
Remove and vacuum seal in bags with hot water shrinkage at 93°C for 2 s
Ripen at 85% relative humidity at 4°C for 60 days or longer, up to 9–12 months, or at 3°C for 2 months then 10°C for 4–7 months, up to 6–9 months

Source: Robinson (1986), Nath (1993), Kosikowski and Mistry (1997), Early (1998), Scott and others (1998), Spreer (1998), Walstra and others (1999).

consumed as cheese or made into a salad dressing. In the manufacturing of blue cheese, as in that of Swiss cheese, salting is accomplished by the application of dry-salting and brining processes. It is characterized by a cream-bleaching step to show off the blue mold filament against a lighter background and by needling of the block of curd so that the mold can spread the blue mold filaments inside the block. It also has a soft and crumbly texture due to the needling process and the gravity draining procedure to drain the curd. The curing period of two to four months is shorter than that for hard cheeses. Its shelf-life of two months is also shorter than that of its harder counterparts. Table 2.16 lists the basic steps in the manufacture of blue cheese (Robinson 1986; Nath 1993; Kosikowski and Mistry 1997; Early 1998; Scott and others 1998; Spreer 1998; Walstra and others 1999).

2.2.2.5 American-Style Camembert Cheese. American-style Camembert cheese is categorized as a soft cheese. It is characterized by having a shell of mold filament on the surface produced by *Penicillium camemberti*. Brie cheese is a similar product. Addition of annatto color is optional. Like blue cheese, it is gravity drained, and it therefore has a soft, smooth texture. This cheese is surface salted and has a total curing period of 3 weeks before distribution. It is usually cut into wedges and wrapped individually for direct consumption. Table 2.17 lists the basic steps in the manufacture of American-style Camembert cheese (Robinson 1986; Nath 1993; Kosikowski and Mistry 1997; Early 1998; Scott and others 1998; Spreer 1998; Walstra and others 1999).

TABLE 2.15 Basic Steps in of Making Swiss Cheese.

Standardize cheese milk to 3% milk fat; treat with H_2O_2-catalase (optional)
Pasteuriz the milk
Inoculate with starters:
Streptococcus thermophilus, 330 ml per 1000 kg milk
Lactobacillus delbrueckkii subsp. bulgaricus, 330 ml per 1000 kg milk
Propionibacterium freudenreichii subsp. shermanii, 55 ml per 1000 kg milk
Add rennet, 10–20 min after inoculation:
154 ml single strength (1 : 15,000) rennet extract per 1000 kg milk, pre-diluted 1 : 40 with tap water before addition
Stir for 3 min
Set (coagulation) of milk in 25–30 min
Cut the curd with 0.61-cm wire knife and let curd stand undisturbed for 5 min and stir at medium speed for 40 min
Cook the curd slowly to 50–53°C for about 30 min and stir at medium speed; then turn off steam and continue stirring for 30–60 min with pH reaching 6.3–6.4
Drip the curd for 30 min
Press the curd with preliminary pressing, then at 69 kPa overnight
First salting: in 23% salt brine for 2–3 days at 10°C
Second salting: at 10–16°C, 90% relative humidity for 10–14 days by wiping the cheese surface from brine soaking followed by sprinkling of salt over cheese surface daily
Third salting: at 20–24°C, 80–85% relative humidity. Wash cheese surface with salt water and sprinkle with dry salt 2–3 times weekly for 2–3 weeks
Rinded block Swiss cheese:
Cure at 7°C or lower (United States) or 10–25°C (Europe) for 4–12 months
Package in container and store at cool temperature
Rindless block Swiss cheese:
Wrap the block or vacuum pack the blocks
Cure the stacked cheese at 3–4°C for 3–6 weeks
Store at cool temperature

Source: Robinson (1986), Nath (1993), Kosikowski and Mistry (1997), Early (1998), Scott and others (1998), Spreer (1998), Walstra and others (1999).

2.2.2.6 Feta Cheese Manufacturing. Feta cheese is a common cheese in Mediterranean countries. It is a soft cheese and is characterized by its brine curing (maturation) process, which is not common in cheese making. Instead, its manufacture is similar to that of sufu (Chinese fermented tofu, see later in this chapter). Like the other soft cheese, the curing period is only 2 to 3 months. Table 2.18 lists the basic steps in the manufacture of feta cheese (Robinson and Tamime 1991).

2.2.3 Yogurt

Yogurt can be considered as a curdled milk product. Plain yogurt is yogurt without added flavor, stabilizer, or coagulant. Its acceptance is limited to those who really enjoy eating it. With the development of technology, other forms of yogurt, such as flavored and sweetened yogurt, stirred yogurt, yogurt drinks, and frozen yogurt, are now available. Its popularity varies from location to location. It is considered a health food when active or live cultures are added to the final product. Table 2.19 lists the basic steps involved in the manufacture of yogurt. Table 2.3 should also be consulted for reference to other ingredients (Chandan and Shahani 1993; Tamime and Robinson 1999).

Most commercially produced yogurt and its products contain sweeteners, stabilizers, or gums (Table 2.20), fruit pieces, natural and synthetic flavors (Table 2.21), and coloring

TABLE 2.16 Basic Steps in Making Blue Cheese.

Milk preparation
  Separate cream and skim milk
  Pasteurize skim milk by high temperature short time (HTST), cool to 30°C.
  Bleach cream with benzoyl peroxide (optional) and heat to 63°C for 30 s
  Homogenize hot cream at 6–9 mPa and then 3.5 mPa, cool and mix with pasteurized skim milk
Inoculate milk with 0.5% active lactic starter at 30°C. Let stand for 1 h
Add rennet: 158 ml single strength (prediluted 1 : 40) per 1000 kg milk and mix well
Coagulate or set in 30 min
Cut curd with 1.6-cm standard wire knife
Cook curd at 30°C followed by 5 min standing and then agitate every 5 min for 1 h. Whey should have 0.11–0.14 titratable acidity.
Draining of whey by gravity for 15 min
Inoculation with *Penicillium roqueforti* spores: 2 kg coarse salt and 28 g *P. roqueforti* spore powder per 100 kg curd followed by thorough mixing. Addition of food-grade lipase optional.
First salting: dip the curd in 23% brine for 15 min followed by pressing or molding at 22°C with turning every 15 min for 2 h and every 90 min for rest of the day
Second salting: on cheese surface every day for 5 days at 16°C, 85% relative humidity
Final dry salting or brine salting in 23% brine for 24–48 h. Final salt concentration about 4%.
Incubate for 6 days at 16°C, 95% relative humidity
Wax and needle air holes or vacuum pack and needle air holes
Mold filament development in air holes at 16°C for 6–8 days
Curing at 11°C, 95% relative humidity, for 60–120 days
Clean and store:
  Strip off the wax or vacuum packaging bag
  Clean cheese, dry, and re-pack in aluminum foil or vacuum packaging bags
  Store at 2°C.
Product shelf-life, 2 months

Source: Robinson (1986), Nath (1993), Kosikowski and Mistry (1997), Early (1998), Scott and others (1998), Spreer (1998), Walstra and others (1999).

TABLE 2.17 Basic Steps in Making American-Style Camembert Cheese.

Standard the milk
Homogenize the milk
Pasteurize the milk at 72°C for 6 s
Cool milk to 32°C
Inoculate with 2% active lactic starter followed by 15–30 min acid ripening to 0.22% titratable acidity
Add annatto color at 15.4 ml per 1000 kg milk (optional)
Add rennet: 220 ml single strength (prediluted 1 : 40) per 1000 ml followed by mixing for 3 min and standing for 45 min
Cut curd with 1.6-cm standard wire knife
Cook curd at 32°C for 15 min with medium-speed stirring
Drain curd at 22°C for 6 h with occasional turning
Inoculate with *Penicillium camemberti* spores using spray gun on both sides of the cheese once
Pressing and mold curd by pressing for 5–6 h at 22°C without any weight on surface
Surface salt the cheese and let stand for about 9 h
Cure at 10°C, 95% relative humidity for 5 days undisturbed, then turn once and continue curing for 14 days
Package, store, and distribute:
  Wrap cheese and store at 10°C, 95–98% relative humidity for another 7 days
  Move to cold room at 4°C and cut into wedges, if required, and re-wrap
  Distribute immediately

Source: Early (1998), Kosikowski and Mistry (1997), Nath (1993), Robinson (1986), Scott and others (1998), Spreer (1998), Walstra and others (1999).

TABLE 2.18 Basic Steps in of Making Feta Cheese.

Standardize milk with 5% fat, enzyme treat and decolorize
Homogenize the milk
Pasteurize by standard procedure and cool to 32°C
Inoculate with 2% active lactic starter as cheddar cheese followed by 1 h ripening
Add rennet at 198 ml single strength (prediluted, 1 : 40) per 1000 kg milk followed by 30–40 min setting
Cut the curd with 1.6-cm standard wire knife followed by 15–20 standing
Drip the curd for 18–20 h at 12–18 kg on 2000 sq. cm with pH and titratable acidity developed to 4.6 and 0.55%, respectively
Prepare cheese blocks of 13 × 13 × 10 cm each
Salt in 23% salt brine for 1 day at 10°C
Can and box the cheese blocks in 14% salt brine (sealed container)
Cure for 2–3 months at 10°C
Soak cured cheese in skim milk for 1–2 days before consumption to reduce salt
Yield: 15 kg/100 kg of 5% fat milk

Source: Robinson and Tamime (1991).

compounds (Table 2.22) (Chandan and Shahani 1993; Tamime and Robinson 1999). Different countries also have different standards on the percentage of fat and solids-not-fat (SNF) in their yogurt products (Table 2.23) (Chandan and Shahani 1993; Tamime and Robinson 1999).

The different variables described above make the situation complicated. The term "yogurt" in one country may not have the same meaning in another country. This also creates difficulties for international trade. Consensus or agreement among countries, and proper labeling, are needed to identify the products properly.

2.2.4 Fermented Liquid Milks

In milk-producing countries, it is common to have fermented milk products. These products were first discovered or developed by accident. Later, the process was modified for commercial production. Fermented liquid milks are similar to plain yogurt drinks, basically milk that has gone through an acid and/or alcoholic fermentation. The final product is maintained in liquid form rather than in the usual soft-gel form of yogurt. There are different fermented liquid milks available, but only sour milk, kefir, and acidophilus milk are discussed below. Readers should refer to the references and other available literature on related products.

TABLE 2.19 Basic Steps in the Production of Yogurt.

Standardize the liquid milk
Homogenize the liquid milk
Heat treat or pasteurize the liquid milk at 90°C for 5 min or equivalent
Cool the pasteurized milk to 1–2°C above inoculation temperature
Add starter (inoculation), 1–3% operational culture
Add flavor, sweetener, gums, and/or color (optional)
Incubate at 40–45°C for 2.5–3.0 h for standard cultures
Break curd (optional)
Cool to 15–20°C in 1–1.5 h
Add live culture (optional)
Package
Store at $\leq$10°C

Source: Chandan and Shahani (1993), Tamime and Robinson (1999).

TABLE 2.20 Some Common Gums that Could be Used in Yogurt Manufacturing.

Kind	Name of Gum
Natural	Agar
	Alginates
	Carageenan
	Carob gum
	Corn starch
	Casein
	Furcelleran
	Gelatin
	Gum arabic
	Guar gum
	Karaya gum
	Pectins
	Soy protein
	Tragacanth gum
	Wheat starch
Modified	Cellulose derivatives
	Dextran
	Low-methoxy pectin
	Modified starches
	Pregelatinized starches
	Propylene glycole alginate
	Xanthin
Synthetic	Polyethylene derivatives
	Polyvinyl derivatives

Source: Chandan and Shahani (1993), Tamime and Robinson (1999).

2.2.4.1 Sour Milk Manufacturing. Table 2.24 presents the basic steps in the manufacture of the most basic fermented liquid milk, sour milk. The milk is standardized, pasteurized, inoculated, incubated, homogenized, and packaged. It is a very straightforward procedure compared to those for the other two products, kefir and acidophilus milk (Davies and Law 1984; Jenness 1988; Robinson 1990; Jay 1996; Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Walstra and others 1999).

TABLE 2.21 Some Common Flavors for Yogurt.

Retail Flavor	Natural Characteristic-Impact Compound	Synthetic Flavoring Compound Available
Apricot	NA	g-Undecalactone
Banana	3-Methylbutyl acetate	NA
Bilberry	NA	NA
Blackcurrant	NA	*trans*- and *cis*-*p*-Methane-8-thiol-3-one
Grape, Concord	Methyl antranilate	NA
Lemon	Citral	15 compounds
Peach	g-Decalactone	g-Undecalactone
Pineapple	NA	Allyl hexanoate
Rasberry	1-*p*-Hydroxyphenyl-3-butanone	NA
Strawberry	NA	Ethyl-3-methyl-3-phenylglycidate

Source: Chandan and Shahani (1993), Tamime and Robinson (1999).

TABLE 2.22 Permitted Yogurt Colorings.

Name of Color	Maximum Level (mg/kg)
Intigotine	6
Brilliant black PN	12
Sunset yellow FCF	12
Tartrazine	18
Cochineal	20
Carminic acid	20
Erythrosine	27
Red 2G	30
Ponceau	48
Caramel	150
Brilliant blue FCF	200

Source: Chandan and Shahani (1993), Tamime and Robinson (1999).

TABLE 2.23 Existing or Proposed Standards for Commercial Yogurt Composition in Selected Countries.

	%Fat			
Country	Low	Medium	Normal	%SNF[a]
Australia	NA	0.5–1.5	3	NA
France	0.5	NA	3	NA
Italy	1	NA	3	NA
Netherlands	1	NA	3	NA
New Zealand	0.3	NA	3.2	NA
UK	0.3	1.0–2.0	3.5	8.5
United States	0.5–1.0	2	3.25	8.5
West Germany	0.5	1.5–1.8	3.5	8.25–8.5
FAO/WHO	0.5	0.5–3.0	3	8.2
Range	0.3–1.0	0.5–3.0	3–3.5	8.2–8.5

[a]%SNF, % solid-not-fat.
Source: Chandan and Shahani (1993), Tamime and Robinson (1999).

TABLE 2.24 Basic Steps in Sour Milk Processing.

Standardize the milk
Heat the milk to 85–95°C followed by homogenization
Cool the milk to 19–25°C and transfer the milk to the fermentation tank
Add 1–2% start culture (inoculation)
Shock-free fermentation to pH 4.65–4.55
Homogenize the gel
Cool to 4–6°C
Filter into bottles, jars, or one-way packs or wholesale packs

Source: Davies and Law (1984), Jenness (1988), Robinson (1990), Jay (1996), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

TABLE 2.25 Basic Steps in Kefir Processing.

Preparation of Mother Kefir
Standardize the milk for preparation of mother "Kefir"
Pasteurize milk at 90–95°C for 15 min and cool to 18–22°C
Spread kefir grains at the bottom of a container (5–10 cm thick) and add pasteurized milk (20–30 times the volume of kefir grains)
Ferment for 18–24 h, mixing 2–3 times. Kefir grains float to the surface
Filter out the kefir grains with a fine sieve; wash the grains with water and save for the next fermentation
Save the fermented milk for the next-step inoculation
Preparation of Drinkable Kefir
Blend fermented milk from above with 8–10 times the volume of fresh, pasteurized, and untreated milk.
Filter into bottles, close and ferment for 1–3 days at 18–22°C.
(Another option is to mix the fermented milk with fresh milk at 1–5%; ferment at 20–25°C for 12–15 h until reaching pH 4.4–4.5, followed by ripening in storage tanks for 1–3 days at 10°C. Product is not as traditional but acceptable.)
Cool to refrigerated temperature
Store and distribute

Source: Davies and Law (1984), Jenness (1988), Robinson (1990), Jay (1996), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

2.2.4.2 Kefir Manufacturing. Kefir is a fermented liquid milk product characterized by the small amount of alcohol it contains, and its inoculant, the kefir grains. It is a common product in Eastern European countries, and is considered to have health benefits. Among all the fermented dairy products, only this and similar products contain a small amount of alcohol. Also, among all other fermented dairy products, pure cultures of

TABLE 2.26 Basic Steps for Processing Sweet Acidophilus Milk.

Procedure #1
Standardize milk
Heat milk to 95°C for 60 min, cool to 37°C and hold for 3–4 h, re-heat to 95°C for 10–15 min, cool to 37°C
Inoculate with 2–5% bulk starter
Incubate for up to 24 h or to 1% lactic acid
Cool to 5°C
Pack and distribute
Procedure #2
Standardize milk
Homogenize milk at 14.5 MPa
Heat to 95°C for 60 min
Cool to 37°C
Inoculate with direct vat inoculation (DVI) starter
Incubate for 12–16 h or to about 0.65% lactic acid
Ready ultra high temperature (UHT) of 140–145°C for 2–3 s to eliminate undesirable contaminants
Cool to 10°C or lower
Package and distribute

Source: Davies and Law (1984), Jenness (1988), Robinson (1990), Jay (1996), Kosikowski and Mistry (1997), Early (1998), Spreer (1998), Walstra and others (1999).

bacteria, yeasts, and/or mold are used, but in kefir, the kefir grains are used and recycled. Kefir grains are masses of bacteria, yeasts, polysaccharides, and other products of bacterial metabolism, together with curds of milk protein. Production of kefir is a two-step process: (1) first the production of mother kefir, and (2) production of the kefir drink. Table 2.25 lists the basic steps in kefir manufacture (Davies and Law 1984; Robinson 1986, 1990; Jenness 1988; Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Farnworth 1999; Jay 1996; Walstra and others 1999).

2.2.4.3 Acidophilus Milk. Acidophilus milk is considered to have probiotic benefits. Like yogurt, it is advertised as having live cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (optional). These live cultures are claimed to provide benefit by maintaining a healthy intestinal microflora. Traditional acidophilus milk contains a considerable amount of lactic acid and is considered to be too sour for regular consumers in some locations. Therefore, a small amount of sugar is added to the final product to make it more palatable. This product is called sweet acidophilus milk. Table 2.26 lists the basic steps in the manufacture of acidophilus milk (Davies and Law 1984; Robinson 1986, 1990; Jenness 1988; Jay 1996; Kosikowski and Mistry 1997; Early 1998; Spreer 1998; Walstra and others 1999).

2.3 MEAT PRODUCTS

2.3.1 Ingredients and Types

Fermented meat products such as ham and sausages have been available to various cultures for centuries. It is interesting to learn that the ways these products are produced are essentially very similar in these different cultures. Besides the meat, nitrite and salt, and sugar (optional), pure cultures are sometimes used, especially in fermented sausages. Microorganisms do not merely provide the characteristic flavor of the products; the lactic acid bacteria also produce lactic and other acids that can lower the pH of the products. Pure cultures are sometimes used in hams to lower the pH and thus inhibit the growth of *Clostridium botulinum*. The raw meat for ham manufacturing is just a large chunk of meat, and it is difficult for microorganisms to penetrate into the center, unless they are injected into the interior. Microbial growth is mainly on the surface and the microbial enzymes are gradually diffused into the center. By contrast, in sausages, the cultures, if used, are mixed with the ingredients (ground or chopped meats) at the beginning and the fermentation is carried out without difficulty. Besides, sausages are much smaller than hams. Table 2.27 lists some of the ingredients used in the manufacture of hams and sausages (Townsend and Olsen 1987; Cassens 1990; Hammes and others 1990; Roca and Incze 1990; Incze 1998; Skrokki 1998; Xiong and others 1999; Huang and Nip 2001; Toldra and others 2001).

2.3.2 Hams

Hams, as indicated earlier, are made from large chunks of meat. In Western cultures, ham is manufactured using either a dry cure and/or a brine cure process, sometimes followed by a smoking process. Tables 2.28 and 2.29 list the basic steps involved with the dry cure and brine cure of hams, respectively. These two processes are similar except for the salting step (Townsend and Olsen 1987; Cassens 1990).

TABLE 2.27 Raw Ingredients for Fermented Meat Products.

Ingredient	Ham	Sausage
Meat		
Pork	Yes	Yes
Beef	No	Optional
Casing	No	Yes
Salt	Yes	Yes
Sugar	Optional	Optional
Starter microorganisms	Optional	Optional
Lactobacillus sakei, L. curvatus, L. plantarum, L. pentosus, L. pentoaceus		
Pediococcus pentosaceus, P. acidilactic		
Staplyococcus xylosus, S. carnosus		
Kocuria varians		
Debaryomyces hansenit		
Candida famata		
Penicillium nagiovense, P. chrysogenum		
Spices	Optional	Optional
Other flavoring compounds	Optional	Optional
Moisture retention salts	Optional	Optional
Preservatives	No	No

Source: Townsend and Olsen (1987), Cassens (1990), Hammes and others (1990), Roca and Incze (1990), Incze (1998), Skrokki (1998), Xiong and others (1999), Huang and Nip (2001), Toldra and others (2001).

Chinese hams are manufactured using a dry curing process. Procedures differ slightly depending on the regions where the hams are made. The most famous Chinese ham is the Jinghua ham made in Central China. Yunan ham from Southern China also has a good reputation. In the old days (without refrigeration facilities during processing, transportation and storage), it is believed that the ham completed its aging process during the transportation and storage stages. Today, with controlled temperature and relative humidity rooms, the hams are produced under controlled conditions. Table 2.30 lists the current process used in China for Jinghua ham (Xiong and others 1999; Huang and Nip 2001).

TABLE 2.28 Basic Steps in Dry Cured Ham Processing.

Prepare pork for dry curing
Mix the proper ratio of ingredients (salt, sugar, nitrite and inocula (optional))
Rub the curing mixture into the meat
Stack the green ham for initial dry curing at 36–40°C
Re-rub the green ham and stack for additional curing at 36–40°C (The ham should be left in the cure for the equivalent of 3 days per pound of meat)
Soak the cured ham for 2–3 h, followed by thorough scrubbing
Place green ham in tight-fitting stocknette and hang in smokehouse to dry overnight
Smoke at about 60 or 80°C with 60% relative humidity for 12–36 h
Cool
Vacuum pack and cool store

Source: Townsend and Olsen (1987), Cassens (1990).

TABLE 2.29 Basic Steps in Brine Cured Ham Processing.

Prepare pork for brine curing
Mix the proper ratio of ingredients (salt, sugar, and nitrite with inocula optional; five gallons of brine for 100 lb meat)
Soak the meat in the prepared brine, or stitch pump the brine into the meat (10% of the original weight of the meat), followed by soaking in the brine for 3–7 days vacuum tumbling or massaging (optional)
Remove the meat from the cover brine and wash
Place green ham in tight-fitting stocknette and hang in smokehouse to dry overnight
Smoke at about 60 or 80°C with 60% relative humidity for 12–36 h
Cool
Vacuum pack and cool store

Source: Towsend and Olsen (1987), Cassens (1990).

TABLE 2.30 Basic Steps in Chinese Jinghua Ham Processing.

Select pork hind leg, 5–7.5 kg
Trim
Salt, 7–8 kg salt per 10 kg ham
Stack and overhaul at 0–10°C for 33–40 days
Wash with cold water and brush
Dry in the sun for 5–6 days
Ferment (cure) for 2–3 months at 0–10°C with harmless green mold developing on surface
Brush off the mold and trim
Age for 3–4 months, maximum 9 months; alternate aging process in temperature-programmable room with 60% relative humidity for 1–2 months
Grade
(Yield: about 55–60%)
Pack and distribute

Source: Xiong and others (1999), Huang and Nip (2001).

2.3.3 Sausages

Many European-type sausages are manufactured using a fermentation process. These sausages have their own characteristic flavors due to the formulations and curing processes used. It is not the intent of this chapter to list the various formulations. Readers should consult the references in this chapter and other references available elsewhere. Commercial inocula are available. Bacteria and some yeasts grow inside the sausage during the ripening period, producing a characteristic flavor. Molds can grow on the surface during storage if the sausages are not properly packaged and stored in the refrigerator. Because these sausages are not sterilized, fermentation is an ongoing process and aged sausages carry a stronger flavor. Table 2.31 lists the basic steps in the manufacture of dry-fermented sausages (Hammes and others 1990; Roca and Incze 1990; Incze 1998; Toldra and others 2001).

TABLE 2.31 Basic Steps in Dry (Fermented) Sausage Processing.

Select meat for processing
Chop and mix chopped meat with spices, seasonings, and inocula at a temperature of about 10°C
Stuff the mixture in suitable casings
Link
Cure or dry for 1–3 months in rooms with temperature, relative humidity, and air circulation regulated according to the type of sausages being produced
Pack and cool store

Source: Hammes and others (1990), Roca and Incze (1990), Incze (1998), Toldra and others (2001).

2.4 FERMENTED CEREAL PRODUCTS (BREADS AND RELATED PRODUCTS)

2.4.1 Types of Products and Ingredients

In wheat-producing countries or areas, baked yeast bread is a major staple in people's diet. This is common in the major developed countries. In other countries, other forms of bread may be the major staple. Baked bread may come in different forms such as regular yeast breads, flat breads, and specialty breads. Today, even retarded (chilled or frozen) doughs are available to meet consumers' preferences for a semblance of home-cooked food. For countries or areas with less available energy, other forms of bread such as steamed bread and boiled breads are available. Fried breads are consumed mainly as breakfast or snack items. Table 2.32 lists some examples of different types of breads (Pyler 1988; Groff and Steinbaecher 1995; Quarooni 1996; Cauvain and Young 1998; Quail 1998; Huang 1999).

Today, as a result of centuries of selection, there are different types of wheat available to suit production environments in various regions with diverse climatic conditions. Wheat used for making bread is hard wheat, soft wheat, or a combination of both, to meet product specifications. Wheat kernels are milled with removal of the bran and germ and further processed into wheat flour. Traditionally, this flour is the major ingredient for baking bread. For some health-conscious consumers, whole-wheat flour is now the flour of choice for making bread. Wheat bran is also added to increase the fiber content of the product. Table 2.33 lists the approximate composition of wheat and some of the common wheat products (Pyler 1988; Groff and Steinbaecher 1995; Cauvain and Young 1998).

In the manufacture of various wheat-based breads and related products, the major ingredients are wheat flour, yeast, sourdough bacteria (optional), salt, and water. Other ingredients vary considerably with type of product. These may be classified grossly as optional ingredients, additives, or processing aids. Each country has its own regulations and requirements. Table 2.34 lists basic ingredients, optional ingredients, additives, and processing aids used in the manufacturing of bread and related products (Pyler 1988; Groff and Steinbaecher 1995; Cauvain and Young 1998).

2.4.2 Regular Bread

Table 2.35 lists the basic steps in bread manufacturing (Pyler 1988; Groff and Steinbaecher 1995; Cauvain and Young 1998). There are three basic processes in

TABLE 2.32 Types of Bread and Related Products.

Type	Examples
Baked breads	
Regular yeast breads	Bread (white, whole wheat or multigrain)
Flat (layered) breads	Pocket bread, croissants
Specialty breads	Sourdough bread, rye bread, hamburger bun, part-baked bread, Danish pastry, stuffed bun
Chilled or frozen doughs	Ready-to-be-baked doughs, retarded pizza doughs, frozen proved dough
Steamed breads	Chinese steam bread (mantou), steamed stuffed bun
Fried breads	Doughnuts
Boiled breads	Pretzels

Source: Pyler (1988), Groff and Steinbaecher (1995), Qarooni (1996), Cauvain and Young (1998), Quail (1998), Huang (1999).

TABLE 2.33 Composition of Wheat, Flour, and Germ.

Material	Moisture (%)	Protein (%)	Fat (%)	Total CHO (%)	Fiber (%)	Ash (%)
Wheat						
Hard red spring	13	14	2.2	69.1	2.3	1.7
Hard red winter	12.5	12.3	1.8	71.7	2.3	1.7
Soft red winter	14	10.2	2	72.1	2.3	1.7
White	11.5	9.4	2	75.4	1.9	1.7
Durum	13	12.7	2.5	70.1	1.8	1.7
Flour, straight						
Hard wheat	12	11.8	1.2	74.5	0.4	0.46
Soft wheat	12	9.7	1	76.9	0.4	0.42
Flour, patent						
Bread	12	11.8	1.1	74.7	0.3	0.44
Germ	11	25.2	10	49.5	2.5	4.3

Source: Pyler (1988), Groff and Steinbaecher (1995), Cauvain and Young (1998).

commercial bread making: the straight dough process, the sponge-and-dough process, and the continuous-baking process. The process used is determined by the manufacturer and the equipment available in the baking plant. Table 2.36 lists the basic steps in the different processes. The major difference is in the way the dough is prepared and handled (Pyler 1988; Groff and Steinbaecher 1995; Cauvain and Young 1998). Because the dough may be prepared in various ways, the amounts of ingredients used differ accordingly. Table 2.37 lists two formulations, comparing the differences in ingredients that arise

TABLE 2.34 Breadmaking Functional Ingredients.

Kind	Examples
Basic ingredient	
Wheat flour	Bread flour, whole wheat flour
Yeast	Compressed yeast, granular yeast, cream yeast, dried yeast, instant yeast, encapsulated yeast, frozen yeast, pizza yeast, deactivated yeast *Saccharomyces cervisiae*, *S. carlsburgenis*, *S. exisguus*
Salt	
Water	
Optional ingredients	Whole wheat flour, gluten, soya flour, wheat bran, other cereals or seeds, milk powder, fat, malt flour, egg, dried fruit, vitamins Sourdough bacteria: *Lactobacillus plantarum*, *L. brevis*, *L. fermentum*, *L. sanfrancisco* Other yeasts
Additives	
Emulsifier	Diacetylated tartaric acid esters of mono- and di-glycerides of fatty acids (DATA esters), sodium stearyl-2-lactylate (SSL), distilled monoglyceride, lecithin
Flour treatment agents	Ascorbic acid, L-cysteine, potassium bromate, potassium iodate, azodicarbonamide
Preservatives	Acetic acid, potassium acetate, sodium diacetate, sorbic acid, potassium sorbate, calcium sorbate, propionic acid, sodium propionate, calcium propionate, potassium propionate
Processing aids	Alpha-amylase, hemicellulose, proteinase, novel enzyme systems (lipases, oxidases, peroxidases)

Source: Pyler (1988), Groff and Steinbaecher (1995), Cauvain and Young (1998).

TABLE 2.35 Basic Steps in Regular or Common Bread Making.

Prepare basic and optional ingredients
Prepare yeast or sourdough for inoculation
Mix proper ingredients to make dough
Ferment
Re-mix dough (optional)
Sheet
Mold and pan
Proof in a temperature- and relative-humidity-controlled chamber
Decoratively cut the dough surface (optional)
Bake, steam, fry, or boil
Cool
Pack
Store

Source: Pyler (1988), Groff and Steinbaecher (1995), Cauvain and Young (1998).

TABLE 2.36 Various Breadmaking Processes.

Straight Dough Baking Process
Weigh out all ingredients
Add all ingredients to mixing bowl
Mix to optimum development
First fermentation, 100 min, room temperature, or at 27°C for 1.5 h
Punch
Second fermentation, 55 min, room temperature, or at 27°C for 1.5 h
Divide
Intermediate proofing, 25 min, 30–35°C, 85% relative humidity
Mold and pan
Final proofing, 55 min at 30–35°C, 85% relative humidity
Bake at 191–232°C for 18–35 min to 100°C internal temperature
Sponge-and-Dough Baking Process
Weigh out all ingredients
Mix part of flour, part of water, yeast and yeast food to a loose dough (not developed)
Ferment 3–5 h at room temperature, or at 21°C for 12–16 h
Add other ingredients and mix to optimum development
Fermentation (floor time), 40 min
Divide
Intermediate proofing, 20 min, 30–35°C, 85% relative humidity, or 27°C for 30 min
Mold and pan
Final proofing, 55 min, 30–35°C, 85% relative humidity
Baking at 191–232°C for 18–35 min to 100°C internal temperature
Continuous-Baking Process
Weigh out all ingredients
Mix yeast, water, and maybe part of flour to form liquid sponge
Add remaining flour and other dry ingredients
Mix in dough incorporator
Fermentation, 2–4 h, 27°C
Pump dough to development chamber
Dough development under pressure of 80 psi
Extrusion within 1 min at 14.5°C and pan
Proof for 90 min
Bake at 191–232°C for 18–35 min to ∼100°C internal temperature

Source: Pyler (1988), Groff and Steinbaecher (1995), Cauvain and Young (1998).

TABLE 2.37 Sample Bread Recipes.

White Pan Bread (*Bulk Fermentation or Straight Dough Process*)

Ingredients	Percent (on flour weight)
Flour	100.0
Yeast	1.0
Salt	2.0
Water	57.0
Optional dough improving ingredients	
Fat	0.7
Soya flour	0.7
Malt flour	0.2

White Pan Bread (*sponge and Dough Process*)

Sponge ingredients	Percent (of total flour)
Flour	25.0
Yeast	0.7
Salt	0.5
Water	14.0
Dough ingredients	**Percent (of total flour)**
Flour	75.0
Yeast	2.0
Salt	1.5
Water	44.0
Optional improving ingredients	
Fat	0.7
Soya flour	0.7
Malt flour	0.2

Source: Pyler (1988), Groff and Steinbaecher (1995), Cauvain and Young (1998).

from differences in the dough preparation processes (Pyler 1988; Groff and Steinbaecher 1995; Cauvain and Young 1998).

2.4.3 Retarded Dough

As indicated earlier, retarded dough is also available to some consumers. This type of dough is more accessible in developed countries as refrigerators and freezers are more common. Dough is prepared so that the fermentation is carefully controlled and the dough is packed inside a container. Storage of this package is also carefully controlled. When the package is opened, consumers can just follow the instructions on the package to bake their own bread. The technology is proprietary to the manufacturers, but there are some guidelines available (Table 2.38) (Pyler 1988; Groff and Steinbaecher 1995; Cauvain and Young 1998).

2.4.4 Flat (Layered) Bread

Flat bread is a general term for bread products that do not rise to the same extent as regular bread. Flat breads are common commodities in Middle Eastern countries and in countries or areas with less accessible energy. In developed countries, flat breads are considered specialty breads. The making of the dough is similar to that for regular bread. However, the dough is

TABLE 2.38 General Guidelines for Retarded Dough Production.

Reduce yeast levels as storage times increase
Keep yeast levels constant when using separate retarders and provers
Reduce yeast levels as the dough radius increases
Reduce yeast levels with higher storage temperatures
The lower the yeast level used, the longer the proof time will be to a given dough piece volume
Yeast levels should not be normally be less than 50% of the level used in scratch production
For doughs stored below $-5^{\circ}C$, the yeast level may need to be increased
Reduce the storage temperature to reduce expansion and weight loss from all dough pieces
Lower the yeast levels to reduce expansion and weight losses at all storage temperatures
Dough pieces of large radius are more susceptible to the effects of storage temperatures
The lower freezing rate achieved in most retarder–provers combined with the poor thermal conductivity of dough can cause quality losses
Prove dough pieces of large radius at a lower temperature than those of small radius
Lower the yeast level in the dough to lengthen the final proof time and to help minimize temperature differentials
Maintain a high relative humidity in proof to prevent skinning

Source: Pyler (1988), Groff and Steinbaecher (1995), Cauvain and Young (1998).

flattened and sometimes layered before it is baked directly inside the hearth or in an oven. Table 2.39 lists the general production scheme for flat breads (Qarooni 1996; Quail 1998).

2.4.5 Croissants and Danish Pastries

Croissants and Danish pastries can be considered products that result from modification of the basic bread-making process. The dough preparation steps are similar, but their ingredients are different. Table 2.40 compares the ingredients used in making croissants and Danish pastries. From this table, it is clear that even within each group, the ingredient formulation can vary considerably, producing a wide variety of available products (Pyler 1988; Groff and Steinbaecher 1995; Cauvain and Young 1998).

2.4.6 Steamed Bread (Mantou)

Steamed bread is common in the Chinese community. Plain steamed bread is consumed as the major staple in the northern provinces of China. However, stuffed steamed breads are

TABLE 2.39 General Production Scheme for Flat Bread.

Ingredient preparation
Mixing of ingredients (dough formation)
Fermentation
Dough cutting and rounding
Extrusion and sheeting (optional)
First proofing
Flattening and layering
Second proofing
Second pressing (optional)
Baking or steaming
Cooling
Packaging and distribution

Source: Quarooni (199A6), Quail (1998).

TABLE 2.40 Formulations for Croissants and Danish Pastries.

Flour (100%)	Croissant	Danish Pastries
Flour	100	100
Salt	1.8–2.0	1.1–1.56
Water	52–55.4	43.6–52
Yeast (compressed)	4–5.5	6–7.6
Shortening	2–9.7	6.3–12.5
Sugar	2–10	9.2–25
Egg	0–24	5–25
Skimmed milk powder	3–6.5	4–6.25
Laminating margarine/butter	32–57	50–64

Source: Pyler (1988), Groff and Steinbaecher (1995), Cauvain and Young (1998).

TABLE 2.41 Basic Steps in Steamed Bread Processing.

Selection of flour and ingredients such as milk powder and sugar (optional)
Mixing of dough
Fermentation:
Full fermentation – 1–3 h
Partial fermentation – 0.5–1.5 h
No-time fermentation – 0 h
Remixed fermentation dough – remixing of fully fermented dough with up to 40% of flour by weight
Neutralization with 40% sodium bicarbonate and remixing
Molding
Proofing at 40°C for 30–40 min (no-time dough)
Steaming for about 20 min
Steamed bread is maintained at least warm to preserve quality

Source: Huang (1999).

consumed as specialty items in various parts of China. Manufacture of steamed bread differs from that of regular bread mainly in the dough solidification process. Regular bread uses a baking process, whereas in steamed bread, steaming is used. Consequently, in steamed bread, there is no brown crust on the bread surface because the temperature used is not high enough to cause the browning reaction. Steamed bread is always consumed hot or held in a steamer, because the bread is soft at this temperature. Sometime steamed bread is deep-fried before consumption. Steamed bread hardens when it cools, making it less palatable. Various procedures are available for production of steamed bread. Table 2.41 lists the basic steps in steamed bread processing in China (Huang 1999).

2.5 FERMENTED SOY PRODUCTS

2.5.1 Types of Products and Ingredients

Soybeans have been available to the Chinese people for centuries, and various fermented soy products have been developed and spread to neighboring countries. These countries further developed their own fermented soy products. Soy sauce originating in China is probably the most famous and widely accepted fermented soy product. The credit for this wide acceptance also goes to the Kikkoman Company from Japan, which has

helped promote the availability of soy sauce worldwide through their marketing strategy. Fermented whole soybeans such as ordinary natto, salted soybeans (e.g., Japanese Hama-natto and Chinese Dou-chi), and tempe (Indonesia); fermented soy pastes (e.g., Japanese miso, and Chinese Dou-pan-chiang); and fermented tofus (e.g., sufu and stinky tofu or chao-tofu of Chinese origin) are more acceptable to ethnic groups. Consumers worldwide are gradually accepting these products through cultural exchange activities. The manufacture of these products varies widely. Table 2.42 summarizes the ingredients needed for the manufacture of common fermented soy products (Ebine 1986; Liu 1986, 1997, 1999; Sugiyama 1986; Winarno 1986; Steinkraus 1996; Yoneya 2003; Teng and others 2004).

TABLE 2.42 Raw Ingredients for Fermented Soy Products.

Ingredient	Soy Sauce	Natto	Soy Nuggets	Soy Paste	Tempe	Soy Cheese	Stinky Tofu
			Major Ingredients				
Soy							
Soy bean	Yes	Yes	Yes	Optional	Yes	Yes	Yes
Soybean flour	Optional	No	No	Yes	No	Optional	Optional
Salt	Yes	Yes	Yes	Yes	No	Yes	No
Wheat	Optional	No	No	No	No	No	No
Rice flour	No	No	No	Optional	No	No	No
			Major Microorganisms				
Mold							
Aspergillus oryzae	Yes	No	Yes	Yes	No	Optional	No
Aspergillus sojae	No	No	No	Optional	No	No	No
Mucor hiemalis, M. silivaticus	No	No	No	No	No	Yes	No
M. piaini	No	No	No	No	No	Yes	No
Actinomucor elegans	No	No	No	No	No	Yes	No
A. repens, A. taiwanensis	No	No	No	No	No	Yes	No
Rhizopus oligosporus	No	No	No	No	Yes	No	No
R. chinesis var. chungyuen	No	No	No	No	No	Yes	No
Bacteria							
Bacillus natto	No	Yes	No	No	No	No	No
Klebsiella pneumoniae	No	No	No	No	Yes	No	No
Bacillus sp.	No	No	No	No	No	No	Yes
Streptococcus sp.	No	No	No	No	No	No	Yes
Enterococcus sp.	No	No	No	No	No	No	Yes
Lactobacillus sp.	No	No	No	No	No	No	Yes
Halophlic yeasts							
Saccharomyces rouxii	Yes	No	Yes	Yes	No	No	No
Torulopsis versatlis	Yes	No	Yes	Yes	No	No	No
Halophilic lactic bacteria							
Pediococcus halophilus	Yes	No	Yes	Yes	No	No	No
Bacillus subtilus	Yes	No	Yes	Yes	No	No	No
Additional flavor added	Optional	No	No	No	No	Optional	No
Preservative added	Optional	No	No	No	No	No	No

Source: Ebine (1986), Liu (1986, 1997, 1999), Sugiyama (1986), Winarno (1986), Steinkraus (1996), Yoneya (2003), Teng and others (2004).

2.5.2 Soy Sauce

There are many types of soy sauce, which differ in the ratio of ingredients (wheat and soybeans), the fermentation and extraction procedures, and the flavoring ingredients (caramel and others) used. However, the procedures for manufacturing are similar. Soy sauce is made by fermenting cooked soybeans in salt or brine under controlled conditions to hydrolyze the soy proteins and starches into smaller flavoring components. The soy sauce is then extracted from the fermented soybeans for standardization and packaging. Table 2.43 Lists a generalized scheme for the manufacture of soy sauce. More detailed information is presented in the references listed in this chapter and in the available literature elsewhere (Elbine 1986; Liu 1986, 1997, 1999; Sugiyama 1986; Yoneya 2003).

2.5.3 Fermented Whole Soybeans

2.5.3.1 Ordinary (Itohiki) Natto. Ordinary natto is a typical Japanese fermented whole soybean product. The sticky mucilageous substance on the surface of soybeans is its major characteristic and is now considered to have health benefits. It is produced by a brief fermentation of cooked soybeans with *Bacillus natto*, and it has a short shelf-life. Table 2.44 lists the basic steps in the manufacture of ordinary natto. For detailed information on ordinary natto, please refer to the references in this chapter (Liu 1997, 1999; Yoneya 2003).

2.5.3.2 Hama-natto and Douchi. Hama-natto is fermented whole soybeans produced in the Hama-matsu area of Japan. Similar products are produced in Japan prefixed with different names taken from the production location. A very similar product in the Chinese culture is "tou-chi", or "dou-chi". It is produced by fermenting the cooked soybeans in salt, brine, or soy sauce, and then drying them as individual beans. Hama-natto includes ginger in the flavoring, whereas the inclusion of ginger flavoring is optional in Dou-chi. Table 2.45 lists the basic steps in the production of Hama-natto and Douchi.

TABLE 2.43 Production Scheme for Soy Sauce.

Selection and soaking of beans
Cooking of clean or defatted soybean, pressurized steam cooking at 1.8 kg/cm^2 for 5 min
Cooling of cooked bean to 40°C
Roasting and crushing of wheat
Mixing of prepared soybeans and wheat
Inoculation with *Aspergillus oryzae* or *sojae*
Incubation of mixture to make starter koji at 28–40°C
Addition of brine (23% salt water) to make moromi (mash)
Inoculation with halophilic yeasts and lactic acid bacteria (optional)
Brine fermentation at 15–28°C
Addition of saccharified rice koji (optional)
Aging of moromi (optional)
Separation of raw soy sauce by pressing or natural gravity
Refining
Addition of preservative and caramel (option)
Packaging and storage

Source: Elbine (1986), Liu (1986, 1997, 1999), Sugiyama (1986), Yoneya (2003).

TABLE 2.44 Production Scheme for Itohiki (Ordinary) Natto.

Cleaning of whole soybean
Washing and soaking at 21–25°C for 10–30 h
Pressurized steam cooking of soybean at 1–1.5 kg/cm^2 for 20–30 min
Draining and cooling of soybean at 80°C
Inoculation with *Bacillus natto*
Mixing and packaging in small packages
Incubation:
40–43°C for 12–20 h, or
38°C for 20 h plus 5°C for 24 h
Final product
Refrigeration to prolong shelf-life

Source: Liu (1997, 1999), Yoneya (2003).

For further information, readers should refer to the references in this chapter and other available literature (Liu 1986, 1997, 1999; Yoneya 2003).

2.5.4 Fermented Soy Pastes

Fermented soy pastes are available in both the Chinese and Japanese cultures and they are made in a similar manner. However, the usage of these two products is quite different.

TABLE 2.45 Production Scheme for Soy Nuggets (Hamanatto and Douchi).

Cleaning of whole soybean
Washing and soaking for 3–4 h at 20°C
Steam cooking of soybean at ambient pressure for 5–6 h, or at 0.8 to 1.0 kg/cm^2 for 30–40 min
Draining and cooling of soybean to 40°C
Addition of alum (optional for douchi)
Mixing with wheat flour (optional for Hamanatto)
Inoculation with *Aspergillus oryzae*
Procedure #1 (*Hamanatto*)
Incubation for 50 h at 30–33°C
Soaking of inoculated soybean in flavoring solution for 8 months
Incubation under slight pressure in closed containers
Procedure #2 (*Douchi*)
Incubation at 35–40°C for 5 days
Washing
Incubation for 5–6 days at 35°C
Removal of beans from liquid for drying
Mixing with ginger soaked in soy sauce (Hamanatto only)
Final product (soy nuggets)
Packaging
Refrigeration to prolong shelf-life (optional)

Source: Liu (1986, 1997, 1999), Yoneya (2003).

TABLE 2.46 Production Scheme for Fermented Soybean Pastes (Miso).

Cleaning of whole soybean
Washing and soaking at 15°C for 8 h
Cooking at 121°C for 45–50 min or equivalent
Cooling and mashing the soybeans
Preparation of soaked, cooked, and cooled rice (optional)
Preparation of parched barley (optional)
Inoculation of rice or barley with *Aspergillus oryzae* (tane-koji, optional)
Mixing of koji and rice or barley mixture
Addition and mixing of salt to koji and rice or barley mixture
Inoculation of halophilic yeasts and lactic acid bacteria (optional)
Packing of mixture (mashed soybean and koji) into fermenting vat with 20–21% salt brine
Fermentation at 25–30°C for 50–70 days
Blending and crashing of ripened miso
Addition of preservative and colorant (optional)
Pasteurization (optional)
Packaging and storage

Source: Ebine (1986), Liu (1986, 1997, 1999), Sugiyama (1986), Steinkraus (1996), Yoneya (2003).

The Japanese use their fermented soy paste, miso, in making miso soup, and to a lesser extent, for example, in marinating/flavoring of fish. Miso soup is common in traditional Japanese meals. The Chinese use their fermented soy paste, dou-pan-chiang, mainly as a condiment in food preparation. Dou-pan-chiang can also be made from wing beans, but this is beyond the scope of this chapter. Table 2.46 lists the basic steps in the manufacture of miso. For detail information on miso and dou-pan-chiang, readers should consult the references for this chapter and other literature available elsewhere (Ebine 1986; Liu 1986, 1997, 1999; Sugiyama 1986; Steinkraus 1996; Yoneya 2003).

2.5.5 Fermented Tofu

2.5.5.1 Sufu or Fermented Soy Cheese. Sufu, or fermented soy cheese, is made by fermenting tofu, which is made by coagulating the soy protein in soymilk with calcium and/or magnesium sulfate. It is similar to feta cheese in its fermentation process. Both products are matured in brine in sealed containers. Some packed sufu contains flavoring ingredients. Table 2.47 lists the basic steps in the manufacture of sufu. For detailed information, readers should refer to the list of reference in this chapter and the other available literature (Liu, 1986, 1997, 1999; Teng and others 2004).

2.5.5.2 Stinky Tofu. Stinky tofu is a traditional Chinese food made by fermenting tofu briefly in "stinky brine" produced by fermenting vegetables under special conditions. The tofu is hydrolyzed slightly during this brief fermentation and develops its characteristic flavoring compounds. When this raw stinky tofu is deep-fried, these compounds volatilize and produce the characteristic stinky odor, thus the name "stinky tofu". It is usually consumed with chilli and soy sauces. Stinky tofu is also steamed with condiments for consumption. Table 2.48 lists the basic steps in the manufacture of stinky tofu. Readers should consult the references in this chapter for further reading (Liu 1986, 1997, 1999; Teng and others 2004).

TABLE 2.47 Production Scheme for Sufu (Chinese Soy Cheese).

Cleaning of whole soybean
Soaking
Grinding with water
Straining through cheesecloth to recover soymilk
Heating to boiling and then cooling
Coagulation of soymilk with calcium and/or magnesium sulfate
Cooled to 50°C
Pressing to remove water (formation of tofu)
Sterilization at 100°C for 10 min in a hot-air oven
Inoculation with *mucor*, *actinomucor*, and/or *rhizopus* sp.
Procedure #1
Incubation in dry form for 2–7 days depending on inocula
Incubation (fermentation in 25–30% salt brine) for 1 month or longer
Brining and aging in small containers with or without addition of alcohol or other flavoring ingredients
Procedure #2
Incubation at 35°C for 7 days until covered with yellow mold
Packing in closed container with 8% brine and 3% alcohol
Fermentation at room temperature for 6–12 months
Final product (sufu or Chinese soy cheese)

Source: Liu (1986, 1997, 1999), Teng and others (2004).

TABLE 2.48 Production Scheme for Stinky Tofu.

Cleaning of whole soybean
Soaking
Grinding with water
Straining through cheesecloth to recover soymilk
Heating to boiling and then cooling
Coagulation of soymilk with calcium and/or magnesium sulfate
Cooling to 50°C
Pressing to remove water (formation of tofu)
Pressing to remove additional water
Soaking in fermentation liquid for 4–20 h at 5–30°C
Fresh stinky tofu ready for frying or steaming
Refrigeration to prolong shelf-life

Source: Liu (1986, 1997, 1999), Teng and others (2004).

2.5.6 Tempe (Tempeh)

Tempe is a traditional, commonly consumed Indonesian food. It is made by fermenting cooked soybeans wrapped in wilted banana leaves or plastic wraps. The mold *Rhizopus oligosporus* produces its mycelia and these mycelia penetrate into the block of soybeans. The mold mycelia also surround the block. This fermentation is similar to molded cheese fermentation. Tempe is gradually being accepted by vegetarians in the West as a nutritious and healthy food. It is generally consumed as a deep-fried product. Table 2.49 lists the basic steps in the production of tempe (Winarno 1986; Liu 1997, 1999; Yoneya 2003).

TABLE 2.49 Production Scheme for Tempe.

Cleaning of whole soybeans
Re-hydration in hot water at 93°C for 10 min
Dehulling
Soaking with or without lactic acid overnight
Boiling for 68 min
Draining and cooling to 38°C
Inoculation with *Rhizopus oligosporus* with or without *Klebsiella pneumonia*
Incubation on trays at 35–38°C, 75–78 % relative humidity for 18 h
Dehydration
Wrapping

Source: Winarno (1986), Liu (1997, 1999), Yoneya (2003).

2.6 FERMENTED VEGETABLES

2.6.1 Types of Products and Ingredients

Fermented vegetables were produced in different cultures, in past times, to preserve harvested vegetables for when they were not available or due to climatic limitations. Some of these products started as traditional cultural foods, but became widely accepted in other cultures. It is interesting that most of these processes are similar. Salt can be used in the production of the product or the salt stock. Natural lactic acid fermentation, to produce enough lactic acid to lower pH, is the major microbial activity in these processes. The added salt and produced lactic acid create an environment that can inhibit the growth of other spoilage microorganisms. Leafy vegetables, fruits (commonly used as vegetables), and roots are used as the raw materials. Starter cultures are occasionally used. Vinegar is used in some products. Chilli pepper and other spices are used in many products. Preservatives may also be used to extend shelf-life after the package is opened. Table 2.50 compares the ingredients used in different fermented vegetable

TABLE 2.50 Raw Ingredients for Fermented Vegetables.

Ingredient	Sauerkraut	Western Pickles	Jalenpo Pappers	Kimchi	Oriental Vegetables
Vegetables					
Head cabbage	Yes	No	No	Optional	Optional
Chinese cabbage	No	No	No	Major	Optional
Mustard green	No	No	No	Optional	Optional
Turnip	No	No	No	Optional	Optional
Jalenpo pepper	No	No	Yes	Optional	Optional
Chilli pepper	No	No	No	Yes	Optional
Pickle/cucumber	No	Yes	No	Optional	Optional
Salt	Yes	Yes	Yes	Yes	Yes
Starter culture (lactic acid bacteria)	Optional	Optional	Optional	No	No
Added vinegar	No	Yes	Yes	No	Optional
Added spices	No	Optional	Optional	Optional	Optional
Other added flavors	No	Yes	No	Optional	Optional
Preservative(s)	No	Optional	Optional	Optional	Optional

Source: Desroiser (1977), Fleming and others (1984), Duncan (1987), Anonymous (1991), Beck (1991), Brady (1994), Chiou (2003), Hang (2003), Lee (2003), Park and Cheigh (2003).

TABLE 2.51 Basic Steps in Sauerkraut Processing.

Selection and trimming of white head cabbage
Coring and shredding of head cabbage to 1/8 in. thick
Salting with 2.25–2.50% salt by weight with thorough mixing
Storage of salted cabbage in vats with plastic cover weighed with water to exclude air in the cabbage
Fermentation at 7–23°C for 2–3 months or longer to achieve an acidity of 2.0% (lactic)
Heating to 73.9°C before filling the cans or jars, followed by exhausting, sealing, and cooling
Storage and distribution

Source: Desroiser (1977), Fleming and others (1984), Brady (1994), Hang (2003).

products (Desroiser 1977; Fleming 1984; Duncan 1987; Anonymous 1991; Beck 1991; Brady 1994; Chiou 2003; Hang 2003; Lee 2003; Park and Cheigh 2003).

2.6.2 Sauerkraut

The term sauerkraut literally means sour (sauer) cabbage (kraut). It is a traditional German fermented vegetable product that has spread to other cultures, and it is used on its own or in food preparations. Its sequential growth of lactic acid bacteria has long been recognized. Each lactic acid bacterium dominates the fermentation until its end product becomes inhibitory for its own development, and creates another environment suitable for another lactic acid bacterium to take over. The fermentation continues until most of the available fermentable sugars are exhausted. The production of sauerkraut is not risk-free, and sanitary precautions must be taken to avoid spoilage. Table 2.51 presents the basic steps in sauerkraut processing (Desroiser 1977; Fleming and others 1984; Anonymous 1991; Hang 2003).

2.6.3 Pickles

Western-style pickles are produced by salting pickling cucumbers in vats in salt stocks for long-term storage, followed by desalting, and bottling in sugar and vinegar, with or without spices. The fermentation is still lactic acid fermentation. However, it is more susceptible to spoilage because air may be trapped inside the slightly wax-coated cucumbers. In the salt-curing of cucumbers, therefore, spoilage can occur, and precautions should be taken to avoid its occurrence. Because of their high acidity and low pH as well as the high salt content, the products are generally mildly heat-treated to sterilize or pasteurize them. Table 2.52 lists the basic steps in the production of Western-style

TABLE 2.52 Basic Steps in Fermented Pickles Processing.

Sizing and cleaning of cucumbers
Preparation of 5% (low salt) or 10% (salt stock) brine
Curing (fermenting) of cucumbers in brine for 1–6 weeks to 0.7–1.0% acidity (lactic) and pH 3.4–3.6 dependent on temperature, with salinity maintained at desirable level (15% for salt stock) (addition of sugar, starter culture and spices are optional)
Recovery of pickles from brine followed by rinsing or de-salting (salt stock)
Grading
Packing of pickles into jars and filling with vinegar, sugar, spices and alum depending on formulation
Pasteurization at 74°C for 15 min followed by refrigerated storage, or exhausting to 74°C at cold point followed by sealing and cooling, or vacuum packing followed by heating at 74°C (cold point) for 15 min and then cooling
Storage and distribution

Source: Desroiser (1977), Fleming and others (1984), Duncan (1987), Anonymous (1991), Beck (1991), Brady (1994).

pickles (Desroiser 1977; Fleming and others 1984; Duncan 1987; Anonymous 1991; Beck 1991; Brady 1994).

2.6.4 Kimchi

Kimchi is a traditional Korean fermented vegetable. Most kimchi is characterized by its hot taste because of the fairly high amount of chilli pepper used in the product and its visibility. However, some kimchi are made without chilli pepper, but with garlic and ginger as well as vegetables and ingredients. This kind of kimchi still has the hot taste, but does not have the red color. Vegetables used in making kimchi vary with its formulations – Chinese cabbage, cucumber, and large turnip are most common. Either chilli pepper or garlic and ginger can be used to provide a hot sensation. Other ingredients may also be added to provide a typical flavor. The fermentation is still lactic acid fermentation. Traditionally, kimchi was made in every household in rural areas of Korea to provide vegetables for the winter when other fresh vegetables were not readily available. Today, it is a big industry in Korea, and kimchi is available all year round. Small South-Korean-made kimchi refrigerators are now available to meet the demands of consumers living in cities in South Korea and other parts of the world. In other parts of the world where Koreans are residents, kimchi is available either as a household item or as a commercial product. Kimchi is usually not heat sterilized after packaging in jars. Pasteurization is optional. It is considered to be perishable and stored refrigerated. Kimchi is now gradually being accepted in other cultures and Koreans are attempting to set standards for handling kimchi. Today, China also exports a considerable amount of kimchi to South Korea. Table 2.53 lists the basic steps in the manufacture of kimchi (Lee 2003; Park and Cheigh 2003).

2.6.5 Chinese Pickled Vegetables

The Chinese people also manufacture a wide range of pickled vegetables. Various kinds of vegetables are used as the raw materials. The fermentation can be either a dry-salting or brining process, depending on the product to be manufactured. However, the fermentation is still lactic acid fermentation. The major difference between Chinese-style pickled vegetable products and the Western-style pickles is that desalting is usually not practiced in the manufacture of Chinese-style pickled vegetables. The desalting process is left to the consumers, if needed. Also, some Chinese-style vegetables are made into intermediate-moisture products, which are not available in the Western-style counterparts.

TABLE 2.53 Basic Steps in Kimchi Processing.

Selection of vegetables (Chinese cabbage, radish, cucumber, or others)
Washing of vegetables
Cutting of vegetables, if necessary
Preparation of 8–15% brine
Immersion of vegetables in brine for 2–7 h to achieve 2–4% salt in vegetable
Rinsing and draining briefly
Addition of seasoning
Fermentation at 0°C to room temperature for about 3 days
Package (can also be conducted before fermentation)
Storage at 3–4°C

Source: Lee (2003), Park and Cheigh (2003).

TABLE 2.54 Basic Steps in Fermented Chinese Vegetables.

Selection and cleaning of vegetables
Cutting of vegetables (optional)
Procedure #1
Wilting of vegetables for 1–2 days to remove moisture
Dry salting of vegetables in layers with weights on top (5–7.5% salt)
Fermentation for 3–10 days
Washing
Drying or pressing of fermented vegetables (optional)
Addition of spices and flavoring compounds
Packaging
Sterilization (optional)
Procedure #2
Wilting of cut vegetables
Hot water rinsing of fermentation container
Filling of the container with cut vegetables
Addition of 2–3% brine and other flavoring compounds (optional)
Ferment at 20–25°C for 2–3 days
Ready for direct consumption or packaging and cool storage

Source: Chiou (2003), Lee (2003).

Table 2.54 lists some of the basic steps in the manufacturing of selected Chinese pickled vegetables (Chiou 2003; Lee 2003).

2.7 APPLICATION OF BIOTECHNOLOGY IN THE MANUFACTURING OF FERMENTED FOODS

With advances in biotechnology, microorganisms with special characteristics for the manufacturing of fermented foods have become available. The most significant example is the approval by the FDA of Chy-Max (chymosin produced by genetic manipulation), used in the production of cheese. Its availability greatly reduces the reliance on chymosin from young calves and generates economic savings. Other products with similar or other properties are also available in the market. Genetically modified lactic acid bacteria and yeasts used in fermented food production are also now available to reduce production costs. Gradual acceptance by consumers is the key to the further development and success of biotechnology (Geisen and Holzapfel 1996; Jay 1996; Kosikowski and Mistry 1997; Scott and others 1998; Spreer 1998; Barrett and others 1999; Henriksen and others 1999; Early 1998; Walstra and others 1999). Readers should refer to the references in this chapter and other references available for further information.

2.8 PROCESS MECHANIZATION IN THE MANUFACTURE OF FERMENTED FOODS

Fermented foods produced by traditional methods are labor-intensive and rely a great deal on the experiences of the manufacturers. The main problem is product inconsistency. In most developed countries, products such as cheeses, yogurts, breads, sausages, and soy sauce are now made by highly mechanized processes in order to standardize the products

(Belderok 2000; Caudill 1993; Dairy and Food Industries Supply Association 1993; Gilmore and Shell 1993; Hamada and others 1991; Iwasaki and others 1992; Kamel and Stauffer 1993; Luh 1995; Muramatsu and others 1993; Prasad 1989). This not only provides product consistency, but also reduces production costs. Consumers benefit from these developments. However, some consumers, even in developed countries, still prefer the traditional products, even at an increased cost, because of their unique product characteristics. There are also fermented products that are still made by traditional or semi-mechanized processes because mechanization processes have not been developed for them.

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3

Food Manufacturing in the United States: Standard Industrial Classification

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3.1 Introduction 87
3.2 Industry Group 201: Meat Products 89
3.2.1 Meat Packing Plants (2011) 89
3.2.2 Sausages and Other Prepared Meat Products (2013) 89
3.2.3 Poultry Slaughtering and Processing (2015) 90
3.3 Industry Group 202: Dairy Products 91
3.3.1 Creamery Butter (2021) 91
3.3.2 Natural, Processed, and Imitation Cheese (2022) 92
3.3.3 Dry, Condensed, and Evaporated Dairy Products (2023) 92
3.3.4 Ice-Cream and Frozen Desserts (2024) 93
3.3.5 Fluid Milk (2026) 93
3.4 Industry Group 203: Canned, Frozen, and Preserved Fruits, Vegetables 94
3.4.1 Canned Specialties (2032) 94
3.4.2 Canned Fruits, Vegetables, Preserves, Jams, and Jellies (2033) 95
3.4.3 Dried and Dehydrated Fruits, Vegetables, and Soup Mixes (2034) 96

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

3.4.4 Pickled Fruits and Vegetables, Vegetable Sauces and Seasonings, and Salad Dressings (2035) 96
3.4.5 Frozen Fruits, Fruit Juices, and Vegetables (2037) 97
3.4.6 Frozen Specialties, Not Elsewhere Classified (2038) 97
3.5 Industry Group 204: Grain Mill Products 98
3.5.1 Flour and Other Grain Mill Products (2041) 98
3.5.2 Cereal Breakfast Foods (2043) 99
3.5.3 Rice Milling (2044) 99
3.5.4 Prepared Flour Mixes and Doughs (2045) 99
3.5.5 Wet Corn Milling (2046) 100
3.5.6 Dog and Cat Food (2047) 101
3.5.7 Prepared Feed and Feed Ingredients for Animals and Fowls, Except Dogs and Cats (2048) 101
3.6 Industry Group 205: Bakery Products 102
3.6.1 Bread and Other Bakery Products, Except Cookies and Crackers (2051) 102
3.6.2 Cookies and Crackers (2052) 103
3.6.3 Frozen Bakery Products, Except Bread (2053) 103
3.7 Industry Group 206: Sugar and Confectionery Products 103
3.7.1 Cane Sugar, Except Refining (2061) 103
3.7.2 Cane Sugar Refining (2062) 104
3.7.3 Beet Sugar (2063) 104
3.7.4 Candy and Other Confectionery Products (2064) 104
3.7.5 Chocolate and Cocoa Products (2066) 105
3.7.6 Chewing Gum (2067) 106
3.7.7 Salted and Roasted Nuts and Seeds (2068) 106
3.8 Industry Group 207: Fats and Oils 106
3.8.1 Cottonseed Oil Mills (2074) 106
3.8.2 Soybean Oil Mills (2075) 106
3.8.3 Vegetable Oil Mills, Except Corn, Cottonseed, and Soybean (2076) 107
3.8.4 Animal and Marine Fats and Oils (2077) 107
3.8.5 Shortening, Table Oils, Margarine, and Other Edible Fats and Oils, Not Elsewhere Classified (2079) 108
3.9 Industry Group 208: Beverages 108
3.9.1 Malt Beverages (2082) 108
3.9.2 Malt (2083) 109
3.9.3 Wines, Brandy, and Brandy Spirits (2084) 109
3.9.4 Distilled and Blended Liquors (2085) 109
3.9.5 Bottled and Canned Soft Drinks and Carbonated Waters (2086) 110
3.9.6 Flavoring Extracts and Flavoring Syrups, Not Elsewhere Classified (2087) 110
3.10 Industry Group 209: Miscellaneous Food Preparations And Kindred 111
3.10.1 Canned and Cured Fish and Seafoods (2091) 111
3.10.2 Prepared Fresh or Frozen Fish and Seafoods (2092) 111
3.10.3 Roasted Coffee (2095) 112
3.10.4 Potato Chips, Corn Chips, and Similar Snacks (2096) 112
3.10.5 Manufactured Ice (2097) 113
3.10.6 Macaroni, Spaghetti, Vermicelli, and Noodles (2098) 113
3.10.7 Food Preparations, Not Elsewhere Classified (2099) 113

3.1 INTRODUCTION

In a modern society, trade and commercial transactions, when reduced to the simplest terms, are made by three parties, willingly or unwillingly: government, business or industry, and consumers. A business manufactures a quality product according to the needs and preferences of consumers. The government monitors the activities of the businesses or firms and incorporates such activities into a database. It uses the information for a variety of reasons, including taxation, safety of consumers and workers in the workplace, technology transfers, economic profiles of various industries, the country's growth in relation to any particular industry, and so on. This approach is practiced in most Western countries in the world. The foundation of this government activity or its database accordingly is to classify manufacturing businesses such as car manufacturers, chemical manufacturers, computer manufacturers, food manufacturers, and so on.

The Standard Industrial Classification (SIC) is an establishment–industry classification system that is prepared by the U.S. government for use by all federal agencies, especially those involved in statistics, labor, consumer safety, and so on. Food manufacturing is included in this classification. The SIC is used, among other functions, in the presentation of state and local area estimates of earnings and employment by industry, records of worker injuries, and so on. It is used by the federal government for estimating in the private sector only, although it is designed to cover both public and private economic activities. To understand food manufacturing in the United States, it is important to know the classification of various establishments engaged in food manufacturing in this industry. This is especially important to a food company, because state agencies also use the classification system to assist its state officials in monitoring the food manufactured by each company in terms of

- Compliance with state standards;
- Product safety for the consuming public; and
- Potential economic fraud involving the products.

In the SIC, establishments are classified by the primary activity in which they are engaged, and each establishment is assigned an industry code. The information is provided in the "SIC Manual", which is distributed by the Occupational Safety and Health Administration (OSHA) of the U.S. Department of Labor. The SIC is divided into divisions A to J, where Division D is "Manufacturing". Division D is further divided into major groups. Group 20 refers to "Food and Kindred Products". This major group includes establishments manufacturing or processing foods and beverages for human consumption, and certain related products such as manufactured ice, chewing gum, vegetable and animal fats and oils, and prepared feeds for animals and fowls. Products described as dietetic are classified in the same manner as nondietetic products (e.g., as candy, canned fruits, cookies). Many other establishments such as ingredient manufacturers (e.g., chemical sweeteners) are classified in other major groups under Division D. Table 3.1 describes the structure of Division D.

TABLE 3.1 SIC Major Industrial Group 20, "Foods and Kindred Products".

Industrial Group	Subgroups
201: Meat products	2011: Meat packing plants
	2013: Sausages and other prepared meat products
	2015: Poultry slaughtering and processing
202: Dairy products	2021: Creamery butter
	2022: Natural, processed, and imitation cheese
	2023: Dry, condensed, and evaporated dairy products
	2024: Ice-cream and frozen desserts
	2026: Fluid milk
203: Canned, frozen, and preserved fruits, vegetables	2032: Canned specialties
	2033: Canned fruits, vegetables, preserves, jams, and jellies
	2034: Dried and dehydrated fruits, vegetables, and soup mixes
	2035: Pickled fruits and vegetables, vegetable sauces and seasonings, and salad dressings
	2037: Frozen fruits, fruit juices, and vegetables
	2038: Frozen specialties, not elsewhere classified
204: Grain mill products	2041: Flour and other grain mill products
	2043: Cereal breakfast foods
	2044: Rice milling
	2045: Prepared flour mixes and doughs
	2046: Wet corn milling
	2047: Dog and cat food
	2048: Prepared feed and feed ingredients for animals and fowls, except dogs and cats
205: Bakery products	2051: Bread and other bakery products, except cookies and crackers
	2052: Cookies and crackers
	2053: Frozen bakery products, except bread
206: Sugar and confectionery products	2061: Cane sugar, except refining
	2062: Cane sugar refining
	2063: Beet sugar
	2064: Candy and other confectionery products
	2066: Chocolate and cocoa products
	2067: Chewing gum
	2068: Salted and roasted nuts and seeds
207: Fats and oils	2074: Cottonseed oil mills
	2075: Soybean oil mills
	2076: Vegetable oil mills, except corn, cottonseed, and soybean
	2077: Animal and marine fats and oils
	2079: Shortening, table oils, margarine, and other edible fats and oils, not elsewhere classified
208: Beverages	2082: Malt beverages
	2083: Malt
	2084: Wines, brandy, and brandy spirits
	2085: Distilled and blended liquors
	2086: Bottled and canned soft drinks and carbonated waters
	2087: Flavoring extracts and flavoring syrups, not elsewhere classified
209: Miscellaneous food preparations and kindred	2091: Canned and cured fish and seafoods
	2092: Prepared fresh or frozen fish and seafoods
	2095: Roasted coffee
	2096: Potato chips, corn chips, and similar snacks
	2097: Manufactured ice
	2098: Macaroni, spaghetti, vermicelli, and noodles
	2099: Food preparations, not elsewhere classified

3.2 INDUSTRY GROUP 201: MEAT PRODUCTS

3.2.1 Meat Packing Plants (2011)

This group covers establishments primarily engaged in the slaughtering, for their own account or on a contract basis for the trade, of cattle, hogs, sheep, lambs, and calves for meat to be sold or to be used on the same premises in canning, cooking, curing, and freezing, and in making sausage, lard, and other products. Also included in this industry are establishments primarily engaged in slaughtering horses for human consumption. Establishments primarily engaged in slaughtering, dressing, and packing poultry, rabbits, and other small game are classified in Industry 2015, and those primarily engaged in slaughtering and processing animals not for human consumption are classified in Industry 2048. Establishments primarily engaged in manufacturing sausages and meat specialties from purchased meats are classified in Industry 2013, and those primarily engaged in canning meat for baby food are classified in Industry 2032. In summary, this subgroup covers

- Bacon, slab and sliced;
- Beef;
- Blood meal;
- Boxed beef;
- Canned meats, except baby foods and animal feeds;
- Corned beef;
- Cured meats;
- Dried meats;
- Frankfurters, except poultry;
- Hams, except poultry;
- Hides and skins, cured or uncured;
- Horsemeat for human consumption;
- Lamb;
- Lard;
- Luncheon meat, except poultry;
- Meat extracts;
- Meat packing plants;
- Meat;
- Mutton;
- Pork;
- Sausages;
- Slaughtering plants; except animals not for human consumption;
- Variety meats, edible organs;
- Veal.

3.2.2 Sausages and Other Prepared Meat Products (2013)

This subgroup includes establishments primarily engaged in manufacturing sausages, cured meats, smoked meats, canned meats, frozen meats, and other prepared meats and

meat specialties, from purchased carcasses and other materials. Prepared meat plants operated by packing houses as separate establishments are also included in this industry. Establishments primarily engaged in canning or otherwise processing poultry, rabbits, and other small game are classified in Industry 2015. Establishments primarily engaged in canning meat for baby food are classified in Industry 2032. Establishments primarily engaged in the cutting up and resale of purchased fresh carcasses, for the trade, (including boxed beef) are classified in Wholesale Trade, Industry 5147. In summary, this subgroup includes

- Bacon, slab and sliced;
- Beef;
- Bologna;
- Calf's-foot jelly;
- Canned meats, except baby foods and animal feeds;
- Corned beef;
- Corned meats;
- Cured meats: brined, dried, and salted;
- Dried meats;
- Frankfurters, except poultry;
- Hams, except poultry;
- Headcheese;
- Lard;
- Luncheon meat, except poultry;
- Meat extracts;
- Meat products: cooked, cured, frozen, smoked, and spiced;
- Pastrami;
- Pigs' feet, cooked and pickled;
- Pork: pickled, cured, salted, or smoked;
- Potted meats;
- Puddings, meat;
- Sandwich spreads, meat;
- Sausage casings, collagen;
- Sausages;
- Scrapple;
- Smoked meats;
- Spreads, sandwich: meat;
- Stew, beef and lamb;
- Tripe;
- Vienna sausage.

3.2.3 Poultry Slaughtering and Processing (2015)

This subgroup includes establishments primarily engaged in slaughtering, dressing, packing, freezing, and canning poultry, rabbits, and other small game, or in manufacturing

products from such meats, for their own account or on a contract basis for the trade. This industry also includes the drying, freezing, and breaking of eggs. Establishments primarily engaged in cleaning, oil treating, packing, and grading of eggs are classified in Wholesale Trade, Industry 5144; and those engaged in the cutting up and resale of purchased fresh carcasses are classified in Wholesale and Retail Trade. In summary, this subgroup covers

- Chickens, processed: fresh, frozen, canned, or cooked;
- Chickens: slaughtering and dressing;
- Ducks, processed: fresh, frozen, canned, or cooked;
- Ducks: slaughtering and dressing;
- Egg albumen;
- Egg substitutes made from eggs;
- Eggs: canned, dehydrated, desiccated, frozen, and processed;
- Eggs: drying, freezing, and breaking;
- Frankfurters, poultry;
- Game, small: fresh, frozen, canned, or cooked;
- Game, small: slaughtering and dressing;
- Geese, processed: fresh, frozen, canned, or cooked;
- Geese: slaughtering and dressing;
- Ham, poultry;
- Luncheon meat, poultry;
- Poultry, processed: fresh, frozen, canned, or cooked;
- Poultry: slaughtering and dressing;
- Rabbits, processed: fresh, frozen, canned, or cooked;
- Rabbits, slaughtering and dressing;
- Turkeys, processed: fresh, frozen, canned, or cooked;
- Turkeys: slaughtering and dressing.

3.3 INDUSTRY GROUP 202: DAIRY PRODUCTS

3.3.1 Creamery Butter (2021)

This subgroup cover establishments primarily engaged in manufacturing creamery butter, and therefore incorporates

- Anhydrous butterfat;
- Butter oil;
- Butter powder;
- Butter, creamery and whey;
- Butterfat, anhydrous.

3.3.2 Natural, Processed, and Imitation Cheese (2022)

This subgroup relates to establishments primarily engaged in manufacturing natural cheese (except cottage cheese), processed cheese, cheese foods, cheese spreads, and cheese analogs (imitations and substitutes). These establishments also produce byproducts such as raw liquid whey. Establishments primarily engaged in manufacturing cottage cheese are classified in Industry 2026, and those manufacturing cheese-based salad dressings are classified in Industry 2035. In summary, subgroup 2022 covers

- Cheese analogs;
- Cheese products, imitation or substitutes;
- Cheese spreads, pastes, and cheese-like preparations;
- Cheese, except cottage cheese;
- Cheese, imitation or substitutes;
- Cheese, processed;
- Dips, cheese-based;
- Processed cheese;
- Sandwich spreads, cheese;
- Whey, raw: liquid.

3.3.3 Dry, Condensed, and Evaporated Dairy Products (2023)

In this subgroup are establishments primarily engaged in manufacturing dry, condensed, and evaporated dairy products. Included in this industry are establishments primarily engaged in manufacturing mixes for the preparation of frozen ice-cream and ice milk, and dairy and nondairy base cream substitutes and dietary supplements:

- Baby formula: fresh, processed, and bottled;
- Buttermilk: concentrated, condensed, dried, evaporated, and powdered;
- Casein, dry and wet;
- Cream substitutes;
- Cream: dried, powdered, and canned;
- Dietary supplements, dairy and nondairy base;
- Dry milk products: whole milk, nonfat milk, buttermilk, and whey;
- Eggnog, canned: nonalcoholic;
- Ice-cream mix, unfrozen: liquid or dry;
- Ice milk mix, unfrozen: liquid or dry;
- Lactose, edible;
- Malted milk;
- Milk, whole: canned;
- Milk: concentrated, condensed, dried, evaporated, and powdered;
- Milkshake mix;
- Skim milk: concentrated, dried, and powdered;
- Sugar of milk;
- Whey: concentrated, condensed, dried, evaporated, and powdered;

- Whipped topping, dry mix;
- Yogurt mix.

3.3.4 Ice-Cream and Frozen Desserts (2024)

This includes establishments primarily engaged in manufacturing ice-cream and other frozen desserts. Establishments primarily engaged in manufacturing frozen bakery products, such as cakes and pies, are classified in Industry 2053. In summary, this subgroup covers

- Custard, frozen;
- Desserts, frozen: except bakery;
- Fruit pops, frozen;
- Ice-cream: e.g., bulk, packaged, molded, on sticks;
- Ice milk: e.g., bulk, packaged, molded, on sticks;
- Ices and sherbets;
- Juice pops, frozen;
- Mellorine;
- Parfait;
- Pops, dessert: frozen flavored ice, fruit pudding and gelatin;
- Pudding pops, frozen;
- Sherbets and ices;
- Spumoni;
- Tofu frozen desserts;
- Yogurt, frozen.

3.3.5 Fluid Milk (2026)

This subgroup covers establishments primarily engaged in processing (e.g., pasteurizing, homogenizing, vitaminizing, bottling) fluid milk and cream, and related products, including cottage cheese, yogurt (except frozen), and other fermented milk. Establishments primarily engaged in manufacturing dry-mix whipped toppings are classified in Industry 2023; those producing frozen whipped toppings are classified in Industry 2038; and those producing frozen yogurt are classified in Industry 2024. This subgroup does include

- Buttermilk, cultured;
- Chocolate milk;
- Cottage cheese, including pot, bakers', and farmers' cheese;
- Cream, aerated;
- Cream, bottled;
- Cream, sour;
- Dips, sour cream based;
- Eggnog, fresh: nonalcoholic;
- Flavored milk drinks;
- Half and half;

- Milk processing (pasteurizing, homogenizing, vitaminizing, bottling);
- Milk production, except farm;
- Milk, acidophilus;
- Milk, bottled;
- Milk, flavored;
- Milk, reconstituted;
- Milk, ultra-high temperature;
- Sour cream;
- Whipped cream;
- Whipped topping, except frozen or dry mix;
- Yogurt, except frozen;

3.4 INDUSTRY GROUP 203: CANNED, FROZEN, AND PRESERVED FRUITS, VEGETABLES

3.4.1 Canned Specialties (2032)

This subgroup of Industry Group 203 includes establishments primarily engaged in canning specialty products, such as baby foods, nationality specialty foods, and soups, except seafood. Establishments primarily engaged in canning seafoods are classified in Industry 2091. So, in summary, subgroup 2032 covers

- Baby foods (including meats), canned;
- Bean sprouts, canned;
- Beans, baked: with or without meat, canned;
- Broth, except seafood: canned;
- Chicken broth and soup, canned;
- Chili con carne, canned;
- Chinese foods, canned;
- Chop suey, canned;
- Chow mein, canned;
- Enchiladas, canned;
- Food specialties, canned;
- Italian foods, canned;
- Macaroni, canned;
- Mexican foods, canned;
- Mincemeat, canned;
- Nationality specialty foods, canned;
- Native foods, canned;
- Pasta, canned;
- Puddings, except meat: canned;
- Ravioli, canned;
- Soups, except seafood: canned;

- Spaghetti, canned;
- Spanish foods, canned;
- Tamales, canned;
- Tortillas, canned.

3.4.2 Canned Fruits, Vegetables, Preserves, Jams, and Jellies (2033)

Establishments included in this subgroup are primarily engaged in canning fruits, vegetables, and fruit and vegetable juices; and in manufacturing catsup and similar tomato sauces, or natural and imitation preserves, jams, and jellies. Establishments primarily engaged in canning seafoods are classified in Industry 2091; those manufacturing canned specialties, such as baby foods and soups, except seafood, are classified in Industry 2032. In summary, this subgroup covers

- Artichokes in olive oil, canned;
- Barbecue sauce;
- Catsup;
- Cherries, maraschino;
- Chili sauce, tomato;
- Fruit butters;
- Fruit pie mixes;
- Fruits, canned;
- Hominy, canned;
- Jams, including imitation;
- Jellies, edible: including imitation;
- Juice, fruit: concentrated–hot pack;
- Juices, fresh: fruit or vegetable;
- Juices, fruit and vegetable: canned or fresh;
- Ketchup;
- Marmalade;
- Mushrooms, canned;
- Nectars, fruit;
- Olives, including stuffed: canned;
- Pastes, fruit and vegetable;
- Preserves, including imitation;
- Purees, fruit and vegetable;
- Sauces, tomato-based;
- Sauerkraut, canned;
- Seasonings (prepared sauces), tomato;
- Spaghetti sauce;
- Tomato juice and cocktails, canned;
- Tomato paste;
- Tomato sauce;

- Vegetable pie mixes;
- Vegetables, canned.

3.4.3 Dried and Dehydrated Fruits, Vegetables, and Soup Mixes (2034)

Establishments primarily engaged in sun-drying or artificially dehydrating fruits and vegetables, or in manufacturing packaged soup mixes from dehydrated ingredients are included in this subgroup. Establishments primarily engaged in the grading and marketing of farm dried fruits, such as prunes and raisins, are classified in Wholesale Trade, Industry 5149. So, this subgroup covers

- Dates, dried;
- Dehydrated fruits, vegetables, and soups;
- Fruit flour, meal, and powders;
- Fruits, sulfured;
- Olives, dried;
- Potato flakes, granules, and other dehydrated potato products;
- Prunes, dried;
- Raisins;
- Soup mixes;
- Soup powders;
- Vegetable flour, meal, and powders;
- Vegetables, sulfured.

3.4.4 Pickled Fruits and Vegetables, Vegetable Sauces and Seasonings, and Salad Dressings (2035)

Establishments primarily engaged in pickling and brining fruits and vegetables, and in manufacturing salad dressings, vegetable relishes, sauces, and seasonings are included here. Establishments primarily engaged in manufacturing catsup and similar tomato sauces are classified in Industry 2033, and those packing purchased pickles and olives are classified in Wholesale or Retail Trade. Establishments primarily engaged in manufacturing dry salad dressing and dry sauce mixes are classified in Industry 2099. In summary, subgroup 2035 relates to

- Blue cheese dressing;
- Brining of fruits and vegetables;
- Cherries, brined;
- French dressing;
- Fruits, pickled and brined;
- Horseradish, prepared;
- Mayonnaise;
- Mustard, prepared (wet);
- Olives, brined: bulk;
- Onions, pickled;

- Pickles and pickle salting;
- Relishes, fruit and vegetable;
- Russian dressing;
- Salad dressings, except dry mixes;
- Sandwich spreads, salad dressing base;
- Sauces, meat (seasoning): except tomato and dry;
- Sauces, seafood: except tomato and dry;
- Sauerkraut, bulk;
- Seasonings (prepared sauces), vegetable: except tomato and dry;
- Soy sauce;
- Thousand Island dressing;
- Vegetable sauces, except tomato;
- Vegetables, pickled and brined;
- Vinegar pickles and relishes;
- Worcestershire sauce.

3.4.5 Frozen Fruits, Fruit Juices, and Vegetables (2037)

Establishments included here are primarily engaged in freezing fruits, fruit juices, and vegetables. These establishments also produce important byproducts such as fresh or dried citrus pulp. In summary, this subgroup covers

- Concentrates, frozen fruit juice;
- Dried citrus pulp;
- Frozen fruits, fruit juices, and vegetables;
- Fruit juices, frozen;
- Fruits, quick frozen and coldpack (frozen);
- Vegetables, quick frozen and coldpack (frozen).

3.4.6 Frozen Specialties, Not Elsewhere Classified (2038)

This subgroup covers establishments primarily engaged in manufacturing frozen food specialties, not elsewhere classified, such as frozen dinners and frozen pizza. The manufacture of some important frozen foods and specialties is classified elsewhere. For example, establishments primarily engaged in manufacturing frozen dairy specialties are classified in Industry Group 202, those manufacturing frozen bakery products are classified in Industry Group 205, those manufacturing frozen fruits and vegetables are classified in Industry 2037, and those manufacturing frozen fish and seafood specialties are classified in Industry 2092. Categories included in this subgroup are

- Dinners, frozen: packaged;
- French toast, frozen;
- Frozen dinners, packaged;
- Meats, frozen;
- Native foods, frozen;

- Pizza, frozen;
- Soups, frozen: except seafood;
- Spaghetti and meatballs, frozen;
- Waffles, frozen;
- Whipped topping, frozen.

3.5 INDUSTRY GROUP 204: GRAIN MILL PRODUCTS

3.5.1 Flour and Other Grain Mill Products (2041)

Establishments primarily engaged in milling flour or meal from grain, except rice, are included here. The products of flour mills may be sold plain or in the form of prepared mixes or doughs for specific purposes. Establishments primarily engaged in manufacturing prepared flour mixes or doughs from purchased ingredients are classified in Industry 2045, and those milling rice are classified in Industry 2044. Those products that are in this subgroup 2041 include

- Bran and middlings, except rice;
- Bread and bread-type roll mixes;
- Buckwheat flour;
- Cake flour;
- Cereals, cracked grain;
- Corn grits and flakes for brewers' use;
- Dough, biscuit;
- Doughs, refrigerated or frozen;
- Durum flour;
- Farina, except breakfast food;
- Flour mills, cereals: except rice;
- Flour mixes;
- Flour: blended, prepared, or self-rising;
- Flour: buckwheat, corn, graham, rye, and wheat;
- Frozen doughs;
- Graham flour;
- Granular wheat flour;
- Grits and flakes, corn: for brewers' use;
- Hominy grits, except breakfast food;
- Meal, corn;
- Milling of grains, dry, except rice;
- Mixes, flour: e.g., pancake, cake, biscuit, doughnut;
- Pancake batter, refrigerated or frozen;
- Pizza mixes and prepared dough;
- Semolina (flour);
- Sorghum grain flour;

- Wheat germ;
- Wheat mill feed.

3.5.2 Cereal Breakfast Foods (2043)

Establishments primarily engaged in manufacturing cereal breakfast foods and related preparations, except breakfast bars, are included in this subgroup. Establishments primarily engaged in manufacturing granola bars and other types of breakfast bars are classified in Industry 2064. In summary, then, this subgroup covers

- Breakfast foods, cereal;
- Coffee substitutes made from grain;
- Corn flakes;
- Corn, hulled (cereal breakfast food);
- Farina, cereal breakfast food;
- Granola, except bars and clusters;
- Hominy grits prepared as cereal breakfast food;
- Infants' foods, cereal type;
- Oatmeal (cereal breakfast food);
- Oats, rolled (cereal breakfast food);
- Rice breakfast foods;
- Wheat flakes.

3.5.3 Rice Milling (2044)

Establishments primarily engaged in cleaning and polishing rice, and in manufacturing rice flour or meal are incorporated here. Other important products of this industry include brown rice, milled rice (including polished rice), rice polish, and rice bran. In summary, the following are included:

- Flour, rice;
- Milling of rice;
- Polishing of rice;
- Rice bran, flour, and meal;
- Rice cleaning and polishing;
- Rice polish;
- Rice, brewers';
- Rice, brown;
- Rice, vitamin and mineral enriched.

3.5.4 Prepared Flour Mixes and Doughs (2045)

This subgroup includes establishments primarily engaged in preparing flour mixes or doughs from purchased flour. Establishments primarily engaged in milling flour from grain and producing mixes or doughs are classified in Industry 2041. So, this subgroup covers

- Biscuit mixes and doughs;
- Bread and bread-type roll mixes;
- Cake flour;
- Cake mixes;
- Dough, biscuit;
- Doughnut mixes;
- Doughs, refrigerated or frozen;
- Flour: blended or self-rising;
- Frozen doughs;
- Gingerbread mixes;
- Mixes, flour: e.g., pancake, cake, biscuit, doughnut;
- Pancake batter, refrigerated or frozen;
- Pancake mixes;
- Pizza mixes and doughs.

3.5.5 Wet Corn Milling (2046)

This subgroup includes establishments primarily engaged in milling corn or sorghum grain (milo) by the wet process, and producing starch, syrup, oil, sugar, and byproducts such as gluten feed and meal. Also included in this industry are establishments primarily engaged in manufacturing starch from other vegetable sources (e.g., potatoes and wheat). Establishments primarily engaged in manufacturing table syrups from corn syrup and other ingredients, and those manufacturing starch-based dessert powders, are classified in Industry 2099. This subgroup therefore covers

- Corn oil cake and meal;
- Corn starch;
- Corn syrup (including dried), unmixed;
- Dextrine;
- Dextrose;
- Feed, gluten;
- Fructose;
- Glucose;
- High fructose syrup;
- Hydrol;
- Meal, gluten;
- Oil, corn: crude and refined;
- Potato starch;
- Rice starch;
- Starch, instant;
- Starch, liquid;
- Starches, edible and industrial;

- Steepwater concentrate;
- Sugar, corn;
- Tapioca;
- Wheat gluten;
- Wheat starch.

3.5.6 Dog and Cat Food (2047)

Establishments primarily engaged in manufacturing dog and cat food from cereal, meat, and other ingredients are included here. These preparations may be canned, frozen, or dry. Establishments primarily engaged in manufacturing feed for animals other than dogs and cats are classified in Industry 2048. This subgroup thus contains just

- Cat food;
- Dog food.

3.5.7 Prepared Feed and Feed Ingredients for Animals and Fowls, Except Dogs and Cats (2048)

Establishments primarily engaged in manufacturing prepared feeds and feed ingredients and adjuncts for animals and fowls, except dogs and cats, are covered. Included in this industry are poultry and livestock feed and feed ingredients, such as alfalfa meal, feed supplements, and feed concentrates and feed premixes. Also included are establishments primarily engaged in slaughtering animals for animal feed. Establishments primarily engaged in slaughtering animals for human consumption are classified in Industry Group 201. Establishments primarily engaged in manufacturing dog and cat foods are classified in Industry 2047. So, this subgroup contains

- Alfalfa, cubed;
- Alfalfa, prepared as feed for animals;
- Animal feeds, prepared: except dog and cat;
- Bird food, prepared;
- Buttermilk emulsion for animal food;
- Chicken feeds, prepared;
- Citrus seed meal;
- Earthworm food and bedding;
- Feed concentrates;
- Feed premixes;
- Feed supplements;
- Feeds, prepared (including mineral): for animals and fowls;
- Feeds, specialty: mice, guinea pigs, minks, etc.;
- Fish food;
- Hay, cubed;
- Horsemeat, except for human consumption;

- Kelp meal and pellets;
- Livestock feeds, supplements, and concentrates;
- Meal, bone: prepared as feed for animals and fowls;
- Mineral feed supplements;
- Oats: crimped, pulverized, and rolled: except breakfast food;
- Oyster shells, ground: used as feed for animals and fowls;
- Pet food, except dog and cat: canned, frozen, and dry;
- Poultry feeds, supplements, and concentrates;
- Shell crushing for feed;
- Slaughtering of animals, except for human consumption;
- Stock feeds, dry.

3.6 INDUSTRY GROUP 205: BAKERY PRODUCTS

3.6.1 Bread and Other Bakery Products, Except Cookies and Crackers (2051)

Establishments primarily engaged in manufacturing fresh or frozen bread and bread-type rolls and fresh cakes, pies, pastries and other similar "perishable" bakery products are included here. Establishments primarily engaged in producing "dry" bakery products, such as biscuits, crackers, and cookies, are classified in Industry 2052. Establishments primarily engaged in manufacturing frozen bakery products, except bread and bread-type rolls, are classified in Industry 2053. Establishments producing bakery products primarily for direct sale on the premises to household consumers are classified in Retail Trade, Industry 5461. In summary, this subgroup includes

- Bagels;
- Bakery products, fresh: bread, cakes, doughnuts, and pastries;
- Bakery products, partially cooked: except frozen;
- Biscuits, baked: baking powder and raised;
- Bread, brown: boston and other, canned;
- Bread, including frozen;
- Buns, bread-type (e.g., hamburger, hot dog), including frozen;
- Buns, sweet, except frozen;
- Cakes, bakery, except frozen;
- Charlotte Russe (bakery product), except frozen;
- Croissants, except frozen;
- Crullers, except frozen;
- Doughnuts, except frozen;
- Frozen bread and bread-type rolls;
- Knishes, except frozen;
- Pastries, except frozen: e.g., Danish, French;
- Pies, bakery, except frozen;

- Rolls, bread-type, including frozen;
- Rolls, sweet, except frozen;
- Sponge goods, bakery, except frozen.

3.6.2 Cookies and Crackers (2052)

Establishments primarily engaged in manufacturing fresh cookies, crackers, pretzels, and similar "dry" bakery products are included. Establishments primarily engaged in producing other fresh bakery products are classified in Industry 2051. So, this subgroup covers

- Bakery products, dry: e.g., biscuits, crackers, pretzels;
- Biscuits, baked: dry, except baking powder and raised;
- Communion wafers;
- Cones, ice-cream;
- Cookies;
- Cracker meal and crumbs;
- Crackers: e.g., graham, soda;
- Matzoths;
- Pretzels;
- Rusk;
- Saltines;
- Zwieback.

3.6.3 Frozen Bakery Products, Except Bread (2053)

Establishments primarily engaged in manufacturing frozen bakery products, except bread and bread-type rolls, are included. Establishments primarily engaged in manufacturing frozen bread and bread-type rolls are classified in Industry 2051. This subgroup therefore covers

- Bakery products, frozen: except bread and bread-type rolls;
- Cakes, frozen: pound, layer, and cheese;
- Croissants, frozen;
- Doughnuts, frozen;
- Pies, bakery, frozen;
- Sweet yeast goods, frozen.

3.7 INDUSTRY GROUP 206: SUGAR AND CONFECTIONERY PRODUCTS

3.7.1 Cane Sugar, Except Refining (2061)

Establishments primarily engaged in manufacturing raw sugar, syrup, or finished (granulated or clarified) cane sugar from sugarcane are covered in this subgroup. Establishments

primarily engaged in refining sugar from purchased raw cane sugar or sugar syrup are classified in Industry 2062. Included, these, are

- Cane sugar, made from sugarcane;
- Molasses, blackstrap: made from sugarcane;
- Molasses, made from sugarcane;
- Sugar, granulated: made from sugarcane;
- Sugar, invert: made from sugarcane;
- Sugar, powdered: made from sugarcane;
- Sugar, raw: made from sugarcane;
- Syrup, cane: made from sugarcane.

3.7.2 Cane Sugar Refining (2062)

Establishments primarily engaged in refining purchased raw cane sugar and sugar syrup are included here, covering

- Molasses, blackstrap: made from purchased raw cane sugar or sugar;
- Refineries, cane sugar;
- Sugar, granulated: made from purchased raw cane sugar or sugar;
- Sugar, invert: made from purchased raw cane sugar or sugar syrup;
- Sugar, powdered: made from purchased raw cane sugar or sugar;
- Sugar, refined: made from purchased raw cane sugar or sugar syrup;
- Syrup, made from purchased raw cane sugar or sugar syrup.

3.7.3 Beet Sugar (2063)

This subgroup includes establishments primarily engaged in manufacturing sugar from sugar beets, and covers

- Beet pulp, dried;
- Beet sugar, made from sugar beets;
- Molasses beet pulp;
- Molasses, made from sugar beets;
- Sugar, granulated: made from sugar beets;
- Sugar, invert: made from sugar beets;
- Sugar, liquid: made from sugar beets;
- Sugar, powdered: made from sugar beets;
- Syrup, made from sugar beets.

3.7.4 Candy and Other Confectionery Products (2064)

Establishments primarily engaged in manufacturing candy, including chocolate candy, other confections, and related products, are included here. Establishments primarily

engaged in manufacturing solid chocolate bars from cacao beans are classified in Industry 2066; those manufacturing chewing gum are classified in Industry 2067; and those primarily engaged in roasting and salting nuts are classified in Industry 2068. Establishments primarily engaged in manufacturing confectionery for direct sale on the premises to household consumers are classified in Retail Trade, Industry 5441. So, this subgroup covers

- Bars, candy: including chocolate-covered bars;
- Breakfast bars;
- Cake ornaments, confectionery;
- Candy, except solid chocolate;
- Chewing candy, except chewing gum;
- Chocolate bars, from purchased cocoa or chocolate;
- Chocolate candy, except solid chocolate;
- Confectionery;
- Cough drops, except pharmaceutical preparations;
- Dates: chocolate covered, sugared, and stuffed;
- Fruit peel products: candied, glazed, glace, and crystallized;
- Fruits: candied, glazed, and crystallized;
- Fudge (candy);
- Granola bars and clusters;
- Halvah (candy);
- Licorice candy;
- Lozenges, candy: nonmedicated;
- Marshmallows;
- Marzipan (candy);
- Nuts, candy covered;
- Nuts, glace;
- Popcorn balls and candy-covered popcorn products.

3.7.5 Chocolate and Cocoa Products (2066)

Establishments primarily engaged in shelling, roasting, and grinding cacao beans for the purpose of making chocolate liquor, from which cocoa powder and cocoa butter are derived, and in the further manufacture of solid chocolate bars, chocolate coatings, and other chocolate and cocoa products are covered here. Also included is the manufacture of similar products, except candy, from purchased chocolate or cocoa. Establishments primarily engaged in manufacturing candy from purchased cocoa products are classified in Industry 2064. This subgroup therefore includes

- Baking chocolate;
- Bars, candy: solid chocolate;
- Cacao bean products: chocolate, cocoa butter, and cocoa;
- Cacao beans: shelling, roasting, and grinding for making chocolate;
- Candy, solid chocolate;

- Chocolate bars, solid: from cacao beans;
- Chocolate coatings and syrups;
- Chocolate liquor;
- Chocolate syrup;
- Chocolate, instant;
- Chocolate, sweetened or unsweetened;
- Cocoa butter;
- Cocoa mix, instant;
- Cocoa, powdered: mixed with other substances.

3.7.6 Chewing Gum (2067)

This subgroup includes establishments primarily engaged in manufacturing chewing gum or chewing gum base, and covers

- Chewing gum;
- Chewing gum base.

3.7.7 Salted and Roasted Nuts and Seeds (2068)

Establishments primarily engaged in manufacturing salted, roasted, dried, cooked, or canned nuts or in processing grains or seeds in a similar manner for snack purposes are covered in this subgroup. Establishments primarily engaged in manufacturing confectionery-coated nuts are classified in Industry 2064, and those manufacturing peanut butter are classified in Industry 2099. So, this subgroup includes establishment making

- Nuts, dehydrated or dried;
- Nuts: salted, roasted, cooked, or canned;
- Seeds: salted, roasted, cooked, or canned.

3.8 INDUSTRY GROUP 207: FATS AND OILS

3.8.1 Cottonseed Oil Mills (2074)

Establishments primarily engaged in manufacturing cottonseed oil, cake, meal, and linters, or in processing purchased cottonseed oil other than into edible cooking oils are included here. Establishments primarily engaged in refining cottonseed oil into edible cooking oils are classified in Industry 2079. So, this subgroup covers

- Cottonseed oil, cake, and meal: made in cottonseed oil mills;
- Cottonseed oil, deodorized;
- Lecithin, cottonseed.

3.8.2 Soybean Oil Mills (2075)

Included in this subgroup are establishments primarily engaged in manufacturing soybean oil, cake, and meal, and soybean protein isolates and concentrates, or in processing purchased

soybean oil other than into edible cooking oils. Establishments primarily engaged in refining soybean oil into edible cooking oils are classified in Industry 2079. Covered then are

- Lecithin, soybean;
- Soybean flour and grits;
- Soybean oil, cake, and meal;
- Soybean oil, deodorized;
- Soybean protein concentrates;
- Soybean protein isolates.

3.8.3 Vegetable Oil Mills, Except Corn, Cottonseed, and Soybean (2076)

Establishments primarily engaged in manufacturing vegetable oils, cake and meal, except corn, cottonseed, and soybean, or in processing similar purchased oils other than into edible cooking oils, are included here. Establishments primarily engaged in manufacturing corn oil and its byproducts are classified in Industry 2046; those that are refining vegetable oils into edible cooking oils are classified in Industry 2079; and those refining these oils for medicinal purposes are classified in Industry 2833. In summary, this subgroup covers

- Castor oil and pomace;
- Coconut oil;
- Linseed oil, cake, and meal;
- Oils, vegetable: except corn, cottonseed, and soybean;
- Oiticica oil;
- Palm kernel oil;
- Peanut oil, cake, and meal;
- Safflower oil;
- Sunflower seed oil;
- Tallow, vegetable;
- Tung oil;
- Walnut oil.

3.8.4 Animal and Marine Fats and Oils (2077)

Included here are establishments primarily engaged in manufacturing animal oils, including fish oil and other marine animal oils, and fish and animal meal, and those rendering inedible stearin, grease, and tallow from animal fat, bones, and meat scraps. Establishments primarily engaged in manufacturing lard and edible tallow and stearin are classified in Industry Group 201; those refining marine animal oils for medicinal purposes are classified in Industry 2833; and those manufacturing fatty acids are classified in Industry 2899. So, this subgroup covers

- Feather meal;
- Fish liver oils, crude;

- Fish meal;
- Fish oil and fish oil meal;
- Grease rendering, inedible;
- Meal, meat and bone: not prepared as feed;
- Meat and bone meal and tankage;
- Oils, animal;
- Oils, fish and marine animal: e.g., herring, menhaden, whale;
- Rendering plants, inedible grease and tallow;
- Stearin, animal: inedible;
- Tallow rendering, inedible.

3.8.5 Shortening, Table Oils, Margarine, and Other Edible Fats and Oils, Not Elsewhere Classified (2079)

Establishments primarily engaged in manufacturing shortening, table oils, margarine, and other edible fats and oils, not elsewhere classified, are covered here. Establishments primarily engaged in producing corn oil are classified in Industry 2046. This subgroup therefore contains

- Baking and frying fats (shortening);
- Cottonseed cooking and salad oil;
- Margarine oil, except corn;
- Margarine, including imitation;
- Margarine butter blend;
- Nut margarine;
- Oil, hydrogenated: edible;
- Oil, partially hydrogenated: edible;
- Oil, vegetable winter stearin;
- Olive oil;
- Peanut cooking and salad oil;
- Shortenings, compound and vegetable;
- Soybean cooking and salad oil;
- Vegetable cooking and salad oil, except corn oil: refined.

3.9 INDUSTRY GROUP 208: BEVERAGES

3.9.1 Malt Beverages (2082)

Establishments primarily engaged in manufacturing malt beverages are included here. Establishments primarily engaged in bottling purchased malt beverages are classified in Industry 5181. So, this subgroup covers

- Ale;
- Beer (alcoholic beverage);

- Breweries;
- Brewers' grain;
- Liquors, malt;
- Malt extract, liquors, and syrups;
- Near beer;
- Porter (alcoholic beverage).

3.9.2 Malt (2083)

Establishments primarily engaged in manufacturing malt or malt byproducts from barley or other grains are included, covering

- Malt byproducts;
- Malt: barley, rye, wheat, and corn;
- Malthouses;
- Sprouts, made in malthouses.

3.9.3 Wines, Brandy, and Brandy Spirits (2084)

Included here are establishments primarily engaged in manufacturing wines, brandy, and brandy spirits. This industry also includes bonded wine cellars that are engaged in blending wines. Establishments primarily bottling purchased wines, brandy, and brandy spirits, but which do not manufacture wines and brandy, are classified in Wholesale Trade, Industry 5182. So, this subgroup covers

- Brandy;
- Brandy spirits;
- Wine cellars, bonded: engaged in blending wines;
- Wine coolers (beverages);
- Wines.

3.9.4 Distilled and Blended Liquors (2085)

This subgroup covers establishments primarily engaged in manufacturing alcoholic liquors by distillation, and in manufacturing cordials and alcoholic cocktails by blending processes or by mixing liquors and other ingredients. Establishments primarily engaged in manufacturing industrial alcohol are classified in Industry 2869, and those only bottling purchased liquors are classified in Wholesale Trade, Industry 5182. Covered then are

- Applejack;
- Cocktails, alcoholic;
- Cordials, alcoholic;
- Distillers' dried grains and solubles;
- Eggnog, alcoholic;
- Ethyl alcohol for medicinal and beverage purposes;
- Gin (alcoholic beverage);

- Grain alcohol for medicinal and beverage purposes;
- Liquors: distilled and blended, except brandy;
- Rum;
- Spirits, neutral, except fruit for beverage purposes;
- Vodka;
- Whiskey: bourbon, rye, scotch type, and corn.

3.9.5 Bottled and Canned Soft Drinks and Carbonated Waters (2086)

Included here are establishments primarily engaged in manufacturing soft drinks and carbonated waters. Establishments primarily engaged in manufacturing fruit and vegetable juices are classified in Industry Group 203; those manufacturing fruit syrups for flavoring are classified in Industry 2087; and those manufacturing nonalcoholic cider are classified in Industry 2099. Establishments primarily engaged in bottling natural spring waters are classified in Wholesale Trade, Industry 5149. In summary, those products covered here include

- Beer, birch and root: bottled or canned;
- Carbonated beverages, nonalcoholic: bottled or canned;
- Drinks, fruit: bottled, canned, or fresh;
- Ginger ale, bottled or canned;
- Iced tea, bottled or canned;
- Lemonade: bottled, canned, or fresh;
- Mineral water, carbonated: bottled or canned;
- Soft drinks, bottled or canned;
- Tea, iced: bottled or canned;
- Water, pasteurized: bottled or canned.

3.9.6 Flavoring Extracts and Flavoring Syrups, Not Elsewhere Classified (2087)

Establishments primarily engaged in manufacturing flavoring extracts, syrups, powders, and related products, not elsewhere classified, for soda fountain use or for the manufacture of soft drinks, and colors for bakers'; and confectioners' use, are contained here. Establishments primarily engaged in manufacturing chocolate syrup are classified in Industry 2066. So, included here are

- Beverage bases;
- Bitters (flavoring concentrates);
- Burnt sugar (food color);
- Cocktail mixes, nonalcoholic;
- Coffee flavorings and syrups;
- Colors for bakers' and confectioners' use, except synthetic;
- Cordials, nonalcoholic;
- Drink powders and concentrates;
- Flavoring concentrates;

- Flavoring extracts, pastes, powders, and syrups;
- Food colorings, except synthetic;
- Food glace, for glazing foods;
- Fruit juices, concentrated: for fountain use;
- Fruit, crushed: for soda fountain use.

3.10 INDUSTRY GROUP 209: MISCELLANEOUS FOOD PREPARATIONS AND KINDRED

3.10.1 Canned and Cured Fish and Seafoods (2091)

Included here are establishments primarily engaged in cooking and canning fish, shrimp, oysters, clams, crabs, and other seafoods, including soups; and those engaged in smoking, salting, drying, or otherwise curing fish and other seafoods for the trade. Establishments primarily engaged in shucking and packing fresh oysters in nonsealed containers, or in freezing or preparing fresh fish, are classified in Industry 2092. So, this subgroup covers

- Canned fish, crustacea, and mollusks;
- Caviar, canned;
- Chowders, fish and seafood: canned;
- Clam bouillon, broth, chowder, juice: bottled or canned;
- Codfish: smoked, salted, dried, and pickled;
- Crab meat, canned and cured;
- Finnan haddie (smoked haddock);
- Fish and seafood cakes: canned;
- Fish egg bait, canned;
- Fish, canned and cured;
- Fish: cured, dried, pickled, salted, and smoked;
- Herring: smoked, salted, dried, and pickled;
- Mackerel: smoked, salted, dried, and pickled;
- Oysters, canned and cured;
- Salmon: smoked, salted, dried, canned, and pickled;
- Sardines, canned;
- Shellfish, canned and cured;
- Shrimp, canned and cured;
- Soups, fish and seafood: canned;
- Stews, fish and seafood: canned;
- Tuna fish, canned.

3.10.2 Prepared Fresh or Frozen Fish and Seafoods (2092)

This subgroup contains establishments primarily engaged in preparing fresh and raw or cooked frozen fish and other seafoods and seafood preparations, such as soups, stews, chowders, fishcakes, crabcakes, and shrimp cakes. Prepared fresh fish are eviscerated or

processed by removal of heads, fins, or scales. This industry also includes establishments primarily engaged in the shucking and packing of fresh oysters in nonsealed containers. Included, then, are

- Chowders, fish and seafood: frozen;
- Crabcakes, frozen;
- Crabmeat picking;
- Crabmeat, fresh: packed in nonsealed containers;
- Fish and seafood cakes, frozen;
- Fish fillets;
- Fish sticks;
- Fish: fresh and frozen, prepared;
- Oysters, fresh: shucking and packing in nonsealed containers;
- Seafoods, fresh and frozen;
- Shellfish, fresh and frozen;
- Shellfish, fresh: shucked, picked, or packed;
- Shrimp, fresh and frozen;
- Soups, fish and seafood: frozen;
- Stews, fish and seafood: frozen.

3.10.3 Roasted Coffee (2095)

Covered here are establishments primarily engaged in roasting coffee, and in manufacturing coffee concentrates and extracts in powdered, liquid, or frozen form, including freeze-dried. Coffee roasting by wholesale grocers is classified in Wholesale Trade, Industry 5149. So, this subgroup relates to

- Coffee extracts;
- Coffee roasting, except by wholesale grocers;
- Coffee, found: mixed with grain or chicory;
- Coffee, instant and freeze-dried.

3.10.4 Potato Chips, Corn Chips, and Similar Snacks (2096)

Establishments primarily engaged in manufacturing potato chips, corn chips, and similar snacks are included here. Establishments primarily engaged in manufacturing pretzels and crackers are classified in Industry 2052; those manufacturing candy-covered popcorn are classified in Industry 2064; those manufacturing salted, roasted, cooked or canned nuts and seeds are classified in Industry 2068; and those manufacturing packaged unpopped popcorn are classified in Industry 2099. This subgroup therefore contains

- Cheese curls and puffs;
- Corn chips and related corn snacks;
- Popcorn, popped: except candy covered;

- Pork rinds;
- Potato chips and related corn snacks;
- Potato sticks.

3.10.5 Manufactured Ice (2097)

Establishments primarily engaged in manufacturing ice for sale are covered in this subgroup. Establishments primarily engaged in manufacturing dry ice are classified in Industry 2813. So, this subgroup includes

- Block ice;
- Ice cubes;
- Ice plants, operated by public utilities;
- Ice, manufactured or artificial: except dry ice.

3.10.6 Macaroni, Spaghetti, Vermicelli, and Noodles (2098)

Establishments primarily engaged in manufacturing dry macaroni, spaghetti, vermicelli, and noodles are covered here. Establishments primarily engaged in manufacturing canned macaroni and spaghetti are classified in Industry 2032, and those manufacturing fried noodles, such as Chinese noodles, are classified in Industry 2099. Included are

- Macaroni and products, dry: e.g., alphabets, rings, seashells;
- Noodles: egg, plain, and water;
- Spaghetti, dry;
- Vermicelli.

3.10.7 Food Preparations, Not Elsewhere Classified (2099)

Included here are establishments primarily engaged in manufacturing prepared foods and miscellaneous food specialties, not elsewhere classified, such as baking powder, yeast, and other leavening compounds; peanut butter; packaged tea, including instant; ground spices; and vinegar and cider. Also included in this industry are establishments primarily engaged in manufacturing dry preparations, except flour mixes, consisting of pasta, rice, potatoes, textured vegetable protein, and similar products that are packaged with other ingredients to be prepared and cooked by the consumer. Establishments primarily engaged in manufacturing flour mixes are classified in Industry Group 204. This subgroup therefore covers

- Almond pastes;
- Baking powder;
- Bouillon cubes;
- Box lunches for sale off premises;
- Breadcrumbs, not made in bakeries;
- Butter, renovated and processed;
- Cake frosting mixes, dry;
- Chicory root, dried;

- Chili pepper or powder;
- Chinese noodles;
- Cider, nonalcoholic;
- Coconut, desiccated and shredded;
- Cole slaw in bulk;
- Cracker sandwiches made from purchased crackers;
- Desserts, ready-to-mix;
- Dips, except cheese and sour cream based;
- Emulsifiers, food;
- Fillings, cake or pie: except fruits, vegetables, and meat;
- Frosting, prepared;
- Gelatin dessert preparations;
- Gravy mixes, dry;
- Honey, strained and bottled;
- Jelly, corncob (gelatin);
- Leavening compounds, prepared;
- Marshmallow creme;
- Meat seasonings, except sauces;
- Molasses, mixed or blended;
- Noodles, fried (e.g., Chinese);
- Noodles, uncooked: packaged with other ingredients;
- Pancake syrup, blended and mixed;
- Pasta, uncooked: packaged with other ingredients;
- Peanut butter;
- Pectin;
- Pepper;
- Pizza, refrigerated: not frozen;
- Popcorn, packaged: except popped;
- Potatoes, dried: packaged with other ingredients;
- Potatoes, peeled for the trade;
- Rice, uncooked: packaged with other ingredients;
- Salad dressing mixes, dry;
- Salads, fresh or refrigerated;
- Sandwiches, assembled and packaged: for wholesale market;
- Sauce mixes, dry;
- Sorghum, including custom refining;
- Spices, including grinding;
- Sugar grinding;
- Sugar, industrial maple: made in plants producing maple syrup;
- Sugar, powdered;
- Syrups, sweetening: honey, maple syrup, sorghum;

- Tea blending;
- Tofu, except frozen desserts;
- Tortillas, fresh or refrigerated;
- Vegetables peeled for the trade;
- Vinegar;
- Yeast.

Section II

Flavors: Food Processing, Product Developments, and Recent Advances

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

4

Food Flavorings: Principles of Applications

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4.1 Classification of Foods Based on Their Natural Flavor Characteristics 120
4.1.1 Natural Flavor Characteristics of Foods in Their Raw State 121
4.1.1.1 Materials with Low Flavor Impact 121
4.1.1.2 Materials with Medium Flavor Impact 121
4.1.1.3 Materials with High Flavor Impact 121
4.1.2 Derived Flavor Characteristics of Foods During Processing 121
4.1.2.1 Foods Devoid of Flavor Compounds in Their Natural State 121
4.1.2.2 Foods Endowed with Flavor Compounds in Their Natural State 122
4.2 Use of Flavorings in Food Processing 122
4.2.1 Criteria for Use of Flavorings 123
4.2.1.1 Demonstrable Need for the Flavor 123
4.2.1.2 Compliance with Legal Requirements 124
4.2.1.3 Compatibility with Other Ingredients 124
4.2.1.4 Ability to Withstand Processing Conditions 124
4.2.1.5 Acceptability to the Consumer 124
4.2.2 Point of Introduction and Form of Use of Flavorings 124
4.3 Effect of Processing Conditions on Flavorings in Processed Food 125
4.3.1 Extremes of Temperature 125
4.3.2 Irradiation 126
4.3.3 Mixing and Contact with Air 126
4.3.4 Extremes of Pressure 126
4.3.5 Mixing Sequence 127
4.3.6 Hydrogen Ion Concentration or pH 127
4.4 Flavor Perception and Flavor Systems 127
4.4.1 Flavor Integers 127

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

4.4.2 Seasoners 128
4.4.3 Enhancers 128
4.4.4 Potentiators 128
4.4.5 Antioxidants 129
References 129

This chapter is an introduction to detailed discussions on the technology and use of flavorings in the main branches of the food industry, ranging from beverages, confections, and bakery products to meat and poultry products, sea foods, and variety meals. Thus, in the ensuing chapters, there will be detailed discussions on the use of flavorings in each of several food product segments. The detailed discussion of each product segment becomes necessary because each product group has its own peculiarities, and only few generalizations can be made. This first discussion will therefore focus on a broad review of the principles guiding the technology and use of flavorings in the food industry in general. In addition, there will be a brief review of certain elements of the flavor system, in particular as they affect flavor perception.

Perhaps we can start by stating an obvious fact: the flavor of food is all-important. And there are several reasons for this. First and foremost, many of the foods we eat are lacking in an intrinsic flavor that is satisfying and definite. Diets based on such foods are therefore unattractive and unappetizing without the addition of flavorings. Second, many of the foods we now eat are fabricated or formulated foods. They are formulated from several foodstuffs having widely differing flavors, and a specific flavoring must be added to either confer a definitive character or intensify one of the flavors already present in the food. Third, food preparation has moved progressively from the kitchen to the factory in the last one or two decades. The more intense institutional and/or factory cooking procedures call for adding back the heat-labile and escaped volatile flavor compounds. Fourth, in modern food marketing, the flavor of the food product has become the main selling point in gaining and keeping customer acceptance. Fifth, the introduction of new flavorings is the most frequently explored route, not only in replacement or rejuvenation of existing products with declining sales, and in extension of existing lines to expand sales, but also in repositioning of existing products and in food product development in general. Sixth, of all the three attribute components of food quality, namely color, flavor, and texture, flavor creates the most avenues for offering variety and originality.

It is therefore little surprise that virtually all foods and other consumer goods are now flavored, ranging from bottled water to cigars. One must then justifiably ponder what principles govern the use of the wide array of flavorings employed in the several thousands of products in which they appear. That is basically the task of this chapter.

4.1 CLASSIFICATION OF FOODS BASED ON THEIR NATURAL FLAVOR CHARACTERISTICS

We have already alluded to the fact that many of the foods we eat are lacking in an intrinsic flavor that is both satisfying and quite definite. This needs further clarification here. A distinction needs to be made between natural flavors of foods in their natural state in contradistinction to derived flavors during and after processing. This distinction is fundamental

to an understanding of the basic principles as well as the philosophy for the use of flavorings in foods. It will therefore be helpful to discuss two broad classifications of biological food materials with respect to: (1) Their natural flavor characteristics in the raw state and (2) their derived flavor characteristics during and after processing.

4.1.1 Natural Flavor Characteristics of Foods in Their Raw State

The natural flavor characteristics of food materials in their raw state are important because of the fact that quite a good number of these foods are eaten in the raw state without being cooked. Moreover, such natural flavor characteristics offer a provisional insight into flavoring requirements during processing. It has therefore been quite useful to classify foods generally based on their flavor characteristics in their raw state.

On the basis of the foregoing, it is generally agreed that foods may be classified into three categories (Heath 1978), namely: (1) Low flavor impact, (2) medium flavor impact, and (3) high flavor impact. The characteristics of the three different groups are given in the following.

4.1.1.1 Materials with Low Flavor Impact. The most striking property of foodstuffs in this category is their less than average flavor characteristic. Furthermore, they are highly nutritious. Examples of such low flavor impact foodstuffs, which may require flavoring during processing, include meat, fish, seafoods, fresh milk, cereal grains, and legumes.

4.1.1.2 Materials with Medium Flavor Impact. The foodstuffs in this category are of adequate flavor. Moreover, they are moderately nutritious. Examples of such foodstuffs include fruits, nuts, mushrooms, and aromatic vegetables like onion, garlic, chives, and leeks. Other examples are members of the *Brassicca* species of the cruciferous vegetables like cabbage and cauliflower.

4.1.1.3 Materials with High Flavor Impact. Foodstuffs in this category are extremely flavorful. However, they are of low or no nutritional value. They are therefore useful mainly as flavorings. Examples of such high flavor impact materials include herbs, spices, and vanilla.

4.1.2 Derived Flavor Characteristics of Foods During Processing

The flavor characteristics derived during and after processing are important, especially for processed foods. Such derived flavor characteristics offer a definitive insight into the nature and intensity of flavoring requirements during and after processing. On the basis of the derived flavor, processed foods may be categorized (Heath 1978) as follows: (1) Foods in which the characteristic flavor compounds are not present in the natural product in the raw state, and (2) foods in which the characteristic flavor compounds are present in the natural product in the raw state. The characteristics of the two different groups are as follows:

4.1.2.1 Foods Devoid of Flavor Compounds in Their Natural State. Food materials that are devoid of the characteristic flavors or flavor compounds in their natural state usually contain precursors of such flavor compounds. Such food materials can be further divided on the basis of the mechanism for formation of the flavor

compounds. The two subgroups are as follows: (1) Those in which flavor is developed by fermentation and/or cooking or some form of heat treatment, and (2) those in which flavor is developed by enzymic action in the cold.

Materials in Which Flavor is Developed by Heat. Materials in this group present an interesting case study, especially from the processor's viewpoint. These are materials whose flavor is developed during the processing operation. As such, the unit operations have to be appropriately sequenced such that the developed flavor compounds are not damaged or volatilized in the subsequent unit operations. If there are deleterious effects either on the chemical integrity or concentration of these flavor compounds, then remedial steps must be taken to restore the flavor integrity of the product. This might be in the form of recovery of the flavor compounds, which are then added back, or completely extant flavorings may be employed. Materials in this category range from meat in which the flavor is developed solely by the agency of heat, to such materials like tea, coffee, cocoa, and vanilla, whose flavors are developed by a combination of "fermentation" and heat. Fermentation in this category of foods often refers to the enzymic oxidation of certain compounds.

Materials in Which Flavor is Developed by Enzymic Action in the Cold. Materials in this group also present an interesting case study, similarly from the processor's viewpoint. These are materials whose flavor is developed also during the processing operation by enzymic action, but usually in the cold without the agency of heat. Examples of such materials include pungent spices like mustard and horseradish, as well as some aromatic vegetables like onion and garlic. However, before the reactions leading to flavor formation can be actuated, there has to be some form of cell rupture or comminuting to bring the enzyme into contact with the substrates. Also, the unit operations have to be appropriately sequenced such that the developed flavor compounds are either not damaged, nor volatilize in subsequent processing.

The characteristic flavor in this category of foods comes primarily from the volatile organic sulfur compounds released by the action of some enzymes on certain nonvolatile, odorless amino acid precursors. Leino (1992), Kuo and Ho (1992) and Sinha and others (1992) have discussed extensively the details of the mechanism of flavor release in onion and the other vegetables in this group.

4.1.2.2 Foods Endowed with Flavor Compounds in Their Natural State. Food materials that are endowed with their characteristic flavors or flavor compounds in their natural state present an interesting class of material as far as sourcing for flavoring is concerned. This is due to the fact that the flavor compounds present in the natural product are usually recoverable by chemical and physical means. Examples are the culinary herbs like garden mint, spices like ginger, and umbelliferous fruits such as coriander and caraway.

4.2 USE OF FLAVORINGS IN FOOD PROCESSING

Having discussed the various schemes for the classification of food materials based on their natural flavor characteristics, the ground is set for a thorough examination of the various criteria guiding the use, form of use, and point of introduction of flavorings in processed foods. However, before going into all that, it needs to be pointed out that the

addition of a flavoring (whether natural or synthetic) to a product can result in any of the following three possibilities (Swaine 1972; Heath 1978):

1. The material achieves or assumes the flavor of the added flavoring. Usually this is the case where either a low flavor-impact food or a material that is lacking in intrinsic flavor is involved. A good example is vanilla flavor in ice-cream.
2. The existing flavor of the material is supplemented, fortified, or intensified by that of the added flavoring. This supplementation is usually called for where a substantial portion of the natural flavor has either been lost or destroyed during processing. A ready example is the action of flavor potentiators such as disodium-5′-inosinate and disodium-5′-guanylate, which are usually added to meat and chicken products. A flavor enhancer such as monosodium glutamate (MSG) also works in this way.
3. The flavor of the material is supplanted, masked or covered up by that of the added flavoring. This is usually the case where a material that has an undesirable flavor is involved. Such is the case in the masking of "boar taint" or "sex odor" in the meat of the sexually mature uncastrated male pig or boar with herbs and spices. The occurrences of such species-specific flavors and odors in meats have been thoroughly discussed by Pearson and others (1994).

4.2.1 Criteria for Use of Flavorings

The use of flavorings in processed foods is determined by a number of factors. These factors are not limited to, but generally include (1) a demonstrable need for the flavor, (2) compliance with legal requirements, (3) compatibility with other ingredients, (4) ability to withstand processing, packaging, storage, distribution, and merchandizing conditions, and (5) acceptability to consumers. These criteria will now be examined in detail.

4.2.1.1 Demonstrable Need for the Flavor. The most basic and perhaps the most important criterion for the use of any flavor additive is that there must be a demonstrable need for the flavoring. This is a condition that can be satisfied very easily because this need can be illustrated by a variety of conditions such as the need to make a low flavor-impact food to be more appetizing, the need to give a definitive character to a food that is fabricated from several component materials, the need to restore the integrity of flavors that have been adversely affected by processing conditions, and the need to cover up the unpalatable taste of bitter pharmaceuticals or nutraceuticals. A reason may be a need to make a nutraceutical more appealing; for example, vitamin C and multivitamin tablets that are coated with a sweet taste. "Nutraceutical" is the term often used to refer to a food, dietary supplement, or biologically active compound that improves health and/or well-being (Cardello and Schutz 2003).

The need for the use of flavoring may be further orchestrated either in response to an identified trend in society or in response to changing public preferences and prejudices. In this regard, it is pertinent to point out that consumers are ever looking to food manufacturers for new and innovative flavors to satisfy their sophisticated demands. Also, it is not out of place for market leaders to study societal trends and do some form of forecasting to be able to deliver flavors ahead of customers' demands.

4.2.1.2 Compliance with Legal Requirements. It is important to bear in mind that flavorings are additives and, as such, must meet all legislative guidelines before being used. That is, they must meet all relevant safety regulations discussed in Section 5.2. The other point that needs to be made is that a demonstrable need for an additive is in fact a legislative requirement.

4.2.1.3 Compatibility with Other Ingredients. The compatibility principle is an exacting one, with very wide ramifications. When we say that flavorings intended for use must be compatible with the other ingredients, this is more than a restatement of the legal guidelines highlighted in Section 5.2. According to the guidelines, an additive must not impart any undesirable flavor, odor, color, or texture to the food. Put another way, it must not make the food unacceptable in any way. A more exacting requirement is that it must not be antagonistic to the food. For instance, the additive must not bind to any of the nutrients or make them unavailable to the body.

Beyond these legal requirements of compatibility, there are other unwritten rules that are known to practitioners. For instance, some flavors go hand in hand with some colors. For example citrus flavor goes along with an orange color to give orange identity to carbonated soft drinks, lemon flavor goes along with a very light green color to give lemon identity to carbonated lemon drinks, and strawberry flavor along with a light red color confers a strawberry identity on imitation strawberry drinks.

4.2.1.4 Ability to Withstand Processing Conditions. Any flavoring added to processed foods must be able to withstand the exacting and often adverse processing conditions such as high temperature, ionizing radiation, subatmospheric pressure or vacuum, and superatmospheric pressures. In other words, the flavoring must be stable and still retain its serving properties after processing. More will be said about this in Section 4.3.

4.2.1.5 Acceptability to the Consumer. The acceptability of the flavoring in the end product is vital to the success of the product. As the whole concept of using flavorings in food revolves around stronger consumer preference and acceptability, it is improbable that a flavoring that meets all the previous criteria will be unacceptable to the consumer. However, in situations where this is the case, the processor has to go back to the drawing board to obtain and present to the discriminating consumer what will be acceptable.

4.2.2 Point of Introduction and Form of Use of Flavorings

The twin issues of the point of introduction and form of use of flavorings are so intertwined that it is not so obvious which one takes precedence. Although the issues are interconnected, the factor that receives the prime consideration depends on the peculiar case at hand.

Perhaps we can start by stating that, depending on the peculiarity of the vector material and the processing conditions, most flavorings can usually be obtained in more than one of the following forms: liquids, powders, dispersions, and encapsulated. Within these broad groupings, there are subdivisions. For instance, a liquid flavoring can be dissolved in a wide array of media such as water, alcohol, or oil. The form that will be used will be dictated by the nature of the food material to which the flavoring is to be added. A few examples will illustrate this point.

Encapsulated flavorings are produced by special techniques in which the volatile aromatic compounds are locked up or otherwise protected by a thin film of water-soluble gum, starch, gelatin, or sugar. Such encapsulated forms are suited for use in dry food mixes such as cake mixes and soup mixes for the following reasons: (1) They are dry, free-flowing and nonhygroscopic powders, and they can therefore be readily incorporated into dry food mixes; (2) they are protected from volatile losses and oxidative changes; and (3) when mixed with water, they disintegrate and liberate the flavoring. On the other hand, oil-based flavorings are usually deployed for uses in fat-based products such as shortenings and margarines.

As for the point of introduction of the flavoring, which is also referred to as the mixing sequence, it is to be noted that most food-processing operations consist of a succession of several unit operations. In some cases, the order of succession of such operations cannot be altered without dire consequences. However, there are several other processing operations in which the order of succession can be rearranged. Whenever this rearrangement is possible, the mixing of the flavoring should be done to avoid the most adverse unit operations; that is, adverse with regard to damage to the flavoring. Even in situations where a rearrangement is not possible, the flavoring can still be added towards the end of the processing operation, thus avoiding undue exposure of the added flavorings to damaging conditions.

Finally, it should be emphasized that, by a careful selection of the form of use and the point of introduction of flavorings, the flavoring strength and quality of the added flavor will be retained during processing, packaging, storage, distribution, and merchandizing conditions.

4.3 EFFECT OF PROCESSING CONDITIONS ON FLAVORINGS IN PROCESSED FOOD

In Section 4.2, it was stressed that any flavoring that is to be used in processed foods must be able to withstand the exacting and often adverse processing conditions. Here, we attempt to outline and briefly discuss those factors that may prevent flavorings from being stable and so prevent them from retaining their serving properties after processing. These processing conditions include (1) extremes of temperature (2) irradiation, (3) mixing and contact with air (4) extremes of pressure, (5) mixing sequence, and (6) hydrogen ion concentration or pH.

4.3.1 Extremes of Temperature

Most flavoring materials are volatile in nature and are also subject to thermal decomposition at elevated temperatures. Many flavor compounds such as allyl isothiocyanate, which is the major pungent flavor compound in mustard and Wasabi, are known to be degraded at temperatures as low as 37°C (Chen and Ho 1998). It is therefore advisable to take precautions against undue exposure of particularly themolabile flavorings to high processing temperatures. Some of these precautions have been discussed in Section 4.2 on the use of flavorings, especially with respect to the point of addition and form of use of flavorings. Other precautions include the use of nonthermal processes such as pulsed electric field (PEF) processing to minimize the loss of flavor, color, and nutrients in fruit juices and other foods alike (Dunn 2001; Min and Zhang 2003; Min and others 2003).

It is also important to mention that thermal processing can induce the formation of flavor compounds in a series of reactions known as the Maillard reaction. This reaction occurs when amino acids are heated in the presence of reducing sugars. Alaimo and others (1992) have presented an excellent discussion on some of these and other volatiles formed in the subsequent Amadori rearrangement.

Apart from susceptibility of flavorings to high temperature, the scientific literature is accented with reports on the effects of low temperatures on some volatile flavor compounds. For instance Usai and others (1992) have reported losses in flavors of essential oil of peels of Thompson navel oranges during cold storage and indicted temperatures below 6°C.

4.3.2 Irradiation

Food irradiation refers to the use of ionizing radiation such as gamma rays, from radioactive elements, and electron beams produced in accelerators, for food preservation. They exert this preservative action through their germicidal effects. Although food irradiation is seen primarily as a preservation method, it can also be employed as a more general unit operation to produce specific desirable effects or changes in foods, such as tenderization in irradiated meat. It is also effective for pathogen control in raw meat.

Ionizing radiations are known, however, to induce water radiolysis (Stewart 2001), leading to the formation of hydroxyl radicals ($^{\bullet}OH$), hydrated electrons (e^{-}), hydrogen atoms ($^{\bullet}H$), hydrogen (H_2), hydrogen peroxide (H_2O_2), and hydrated protons (H_3O^{+}). These intermediates are known to react with both organic and inorganic compounds within the food material to produce compounds that are responsible for off-odors in irradiated foods (Ahn and others 2001; Ahn and Lee 2002; Lee and Ahn 2003). Specifically, these intermediates have been implicated in the formation of many distinctive off-flavors in foods, such as "bloody and sweet", "hot fat", and "burnt off" off-odors in meats (Ahn and others 2000), as well as "medicinal" and "cooked off" off-odors in orange juice (Yoo and others 2003). These off-odors impact negatively on the consumer acceptability of these products.

4.3.3 Mixing and Contact with Air

Mixing and, especially high-speed mixing, promotes intimate contact of the food matrix with air, and so predisposes the volatile flavoring in food to oxidation. Losses of flavor volatiles could be appreciable in such high-speed mixing operations. This is especially true in ice-cream making, whipping of eggs, in marshmallows, and to some extent in mechanical dough development during bread making.

Another important unit operation, that promotes aeration of the food material, is the pneumatic or fluidized handling of powders such as soup mixes, cocoa and chocolate beverage powders, cake mixes, and dessert powders. Volatile losses in these products can be minimized through the use of appropriate forms of flavoring, such as encapsulated flavoring.

4.3.4 Extremes of Pressure

Although application of either superatmospheric or subatmospheric pressures can have deleterious effects on the integrity of flavor compounds, negative pressure appears to be implicated more in food processing. This is because negative pressure results in greater losses of volatiles. There are many food processing equipments operating at

subatmospheric pressures, ranging from driers and filters to centrifuges, and many more. Ironically, processing under vacuum is done to mitigate some of the negative consequences of processing at high temperature, including thermal degradation and volatilization of flavor compounds.

4.3.5 Mixing Sequence

In the discussion on the use of flavorings in food processing in Section 4.2.2, we have already highlighted the significance of the point of introduction (also referred to as the mixing sequence) of the flavoring, in avoiding undue exposure of the added flavorings to damaging conditions.

4.3.6 Hydrogen Ion Concentration or pH

Many flavors and flavor compounds are known to be sensitive to fluctuations in pH. In fact, many flavor compounds are known to be more predisposed to chemical transformations, thermal degradation, and other unwanted changes at certain pH ranges or hydrogen ion concentrations. For example, the major pungent flavor compound in mustard, allyl isothiocyanate, is reported to be sensitive to pH. Its decomposition to other compounds having a garlic-like odor in the presence of water at 37°C is known to be accelerated under alkaline conditions (Chen and Ho 1998). Such unfavorable pH conditions should be avoided as much as possible in the processing and handling of such sensitive materials. Furthermore, food-grade buffer systems could be employed where practicable (Turhan and others 2003).

4.4 FLAVOR PERCEPTION AND FLAVOR SYSTEMS

Flavor perception in food systems is built upon the integration of several aroma and oral attributes of the food. The overall flavor is therefore a combination of taste, feeling, and odor on receptors in the oral (mouth and throat) and nasal (nose) cavities. The general flavors discussed in this volume are perceived the way they are because of the influence of certain "silent" elements of the flavor system. A general review of the broad principles guiding the use of flavorings in the food industry will therefore not be complete without a brief on these elements. These silent elements are in fact elements of the stimuli that affect flavor perception, and are generally classified according to the way they affect the particular food or flavor system. They can be listed either as integers, seasoners, enhancers, potentiators, or antioxidants (Sjöström 1972). We shall now discuss each of the elements.

4.4.1 Flavor Integers

Most food matrices consist of water and a rather diverse admixture of other chemical constituents. The major chemical constituents of most foods include carbohydrates, proteins, fats, and their derivatives. In addition, there are inorganic mineral components and a diverse group of organic substances that are present in relatively small quantities. Such organic substances include vitamins, enzymes, emulsifiers, acids, oxidants, antioxidants, pigments, and flavor compounds. These constituents, which have unique arrangements

within each food matrix, give the particular food their peculiar characteristics, such as structure, texture, flavor, color, and nutritive value.

Some of these chemicals combine to produce the stimuli that are then perceived as flavor. Such chemicals that are integral parts of food matrices and at the same time interact to produce flavor sensations are referred to as flavor integers. Sugars, organic acids, and some essential oils are good examples of flavor integers. Many of these integers are present in foods of medium flavor impact like fruits. In many fruits, for instance, and especially the climacteric fruits, ripening is associated with conversion of starch into sugar, which reacts directly on our taste buds to produce the sensation of sweetness. Furthermore, many fruits contain "signature" organic acids such as citric acid in oranges and lemon, tartaric acid in grapes, and malic acid in apples. These organic acids contribute to the peculiar flavor in these fruits, and are therefore invaluable in the formulation of particular imitation fruit drinks. In addition to being flavor enhancers, these acids give the fruits tartness and also slow down bacterial spoilage.

Also, certain essential oils, which are components of essences of many spices, have their individual characters and are powerful flavor factors in their own right. A very good example is eugenol, which accounts for 70–90% of the essential oils of the clove bud, and is generally associated with clove flavor.

4.4.2 Seasoners

Seasoners are so called because they are used for seasoning or altering the aroma and oral attributes of the food in desirable ways. Table salt is undoubtedly the most commonly employed seasoner. It is capable of altering both the aroma as well as the taste of food, and if added to food in high concentrations can also alter the mouth feel.

Other popular seasonings widely used in food processing include the aromatic vegetables of the *Allium* species of the family of Liliaceae. This includes onion, garlic, leeks, chives, scallions, and shallots. The major form of use of these aromatic vegetables in the food industry is the steam distilled flavor or oil.

4.4.3 Enhancers

Flavor enhancers are chemical compounds or materials that are used in very minute quantities to intensify or enhance the flavor that is already present in the food. At the level of parts per thousand, in which they are usually used, they do not add any flavor of their own to the food. The best known of the flavor enhancers is monosodium glutamate (MSG). Other less prominent flavor enhancers, which are similar to MSG, include tricholoric acid and ibotenic acid. These compounds were isolated from some fungi by Japanese scientists.

Monosodium glutamnate is particularly effective in its ability to enhance or intensify the flavor of many high-protein foods. It is not without character. When used in large enough quantities, MSG can contribute a taste of its own, sometimes described as sweet–saline, to the food in which it is added. In such cases, MSG becomes a seasoning.

4.4.4 Potentiators

Potentiation is an action by which a chemical agent by itself and in small quantities has no effect on a biological system, but exaggerates the effects of other chemical reagents on that

system. Flavor potentiators thus work in the same way as flavor enhancers by enhancing or intensifying the flavor of the food to which they are added, without adding any flavor of their own. Flavor potentiators are, however, more intriguing. Prominent flavor potentiators are the 5′-nucleotides such as disodium-5′-inosinate and disodium-5′-guanylate.

Potentiators are different from flavor enhancers in five important respects. (1) Flavor potentiators are much more powerful in that they are effective in concentrations of parts per billion or even much less. (2) At whatever level of use, flavor potentiators do not contribute a taste of their own to the food materials to which they are added. Thus, potentiators can never double as seasoners under any circumstance. (3) The potentiators will consistently intensify certain specific flavor notes regardless of the food to which they are added. (4) The potentiators are stable towards cooking and can be added to food to be cooked. (5) The potentiators are less hygroscopic than enhancers.

From the flavorist's point of view, it is pertinent to point out that there is a specific synergistic action between flavor enhancers and flavor potentiators. This is because the two groups of compounds affect different aroma and oral flavor notes. Flavor potentiators enhance meaty and broth notes, but buttery and sweet notes are not affected. Flavor enhancers intensify mostly attributes of mouth feel such as dryness and astringency. This synergism between the flavor enhancers and flavor potentiators furnishes one of the most important keys to the development of flavor and flavorings in food product development.

4.4.5 Antioxidants

Antioxidants constitute one of the most important but often ignored elements of the flavor system. This is due to the fact that volatile flavor compounds such as aldehydes, ketones, and keto esters are susceptible to oxidation. This, in effect, means that flavorings or food products containing these volatiles lose their flavor during storage. Thus, when an antioxidant like ascorbic acid is added to a food or beverage, it is preferentially oxidized and lost, thereby preserving the major flavor compounds.

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5

Product Development

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5.1 Why New Product Development? 132
5.1.1 When is a New Product Really New? 134
5.2 An Overview of Legislative Guidelines on New Product Development 135
5.2.1 Legislative Guidelines on Food Product Development 136
5.2.1.1 Guidelines on Food Additives 136
5.2.1.2 Regulations on Adulteration and Misbranding 137
5.2.1.3 Labeling of Genetically Modified Foods 138
5.2.2 The Codex Alimentarius Commission 138
5.3 Flavor Creation and New Product Development 139
5.3.1 Generation of New Product Ideas 139
5.3.2 Isolation, Identification, and Synthesis of Flavor Compounds 139
5.3.2.1 Isolation and Concentration of Volatiles 140
5.3.2.2 Identification of Compounds 141
5.3.2.3 Synthesis of Flavor Compounds 141
5.3.3 Formulation and Compounding of Synthetic Flavorings 143
5.3.3.1 Sensory Analysis of Flavorings 143
5.3.3.2 Compounding of Imitation Flavoring 144
5.3.4 Trial Use of Synthetic Flavorings 145
5.3.5 Marketing of the New Product 145
References 145

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

Product development within the context of flavor technology could be construed strictly in terms of creation and development of flavorings. And this will be correct. However, from the wider food industry perspective, it will be apposite to include new flavored food products. Thus, in this chapter, the topic of product development is discussed from the wider food industry perspective, but with special reference to flavor creation.

All flavorings and other food additives, food products, as well as other consumer goods for commercial use, including medicines and cosmetics, are strictly regulated by the enforcement of various legislations and standards. Thus a brief review of the laws and regulations guiding the development, processing and marketing of such food additives, food products, and other consumer goods will be undertaken here.

This review as a prelude to a discussion of the subject matter is important for the simple fact that legislative requirements constitute the most important impediment that tends to slow down innovation and new product development in the consumer industry in general and the food and beverage industry in particular. Thus, due to the strict legal requirements for new additives, the uses of new flavorings are reflected very slowly in end products.

In discussing product development, we shall examine the need for new products in the flavor, as well as the larger food industry. Next, we undertake a discussion of some legislative aspects that have a direct bearing on the success of newly developed products, before finally treating flavor creation and development.

5.1 WHY NEW PRODUCT DEVELOPMENT?

There are many compelling reasons for new product development (Valentas and others 1991). We need to emphasize that the primary purpose of investing capital in plants and equipment for a manufacturing concern is to earn an amount of money that exceeds the investment. In other words, people go into business to make profit. Therefore, one of the most important reasons for new product development is the imperative to constantly replace or rejuvenate existing products that have declining sales. This is with the view of generating new sales volume, meeting projected returns on the investment, and ultimately achieving corporate growth.

In relation to declining sales, it is important to point out that all flavorings, food products, and other consumer goods have a finite life span, just like any living organism. Interestingly, the typical life cycle of any product can be divided into four distinct stages, namely product introduction, market growth, market maturity, and product decline (Albrecht 1981). Suffice it to say that each of these phases has its own characteristic vigor, which is reflected in sales volume and profits. The key point to be made here is that for a flavor manufacturing company or any company for that matter to be successful, it must study the life cycle of its products and know when to introduce new products. Thus, in a fiercely competitive market, new products are what keep companies alive and vital. A company without a viable product-planning profile cannot grow, and will in fact go under sooner rather than later.

Another compelling reason for new product development is the need to take advantage of newer and better technologies. For instance, Blue Pacific Flavors, a leading flavor manufacturing firm in the United States, has developed a new flavor technology, which it refers to as the sun-ripened flavor system (Anonymous 2004a). This system is a proprietary water-soluble, heat-stable matrix that provides intense aroma and taste without the use of traditional solvents like propylene glycol, alcohol, and glycerin. This innovation is bound to create new opportunities in the flavor industry, and before long, similar new

products developed by other firms to take advantage of these new opportunities will start appearing in the market.

Apart from the technology-driven imperative, product development could occur in response to competition from one or more rival products or companies. Usually, whenever a leading manufacturing firm introduces a new product, there is always an upbeat in innovative imitation activities by the other major players in the industry. This phenomenon has been aptly tagged "reverse R&D" (Desrosier and Desrosier 1971). Reverse R&D refers to the efforts of the other manufacturers in trying to create their own imitative equivalents of the innovative products created by the market leaders. Failure of the other manufacturers to make reverse R&D commitments could result in the dipping of their market shares below profitability levels.

Product development could also be in response to identified societal trends and customer needs. This is where the marketing people come in, because they are the ones in contact with the customers and they obtain direct feedback. So, they are in a vantage position to know what the consumers want. Even if the consumers do not voice their opinions, it is part of marketing strategy to feel out the consumer needs and understand the forces that motivate their purchases. In so doing, improvements or innovative products that will sell can be developed and marketed well ahead of the other competitors.

One leading flavor manufacturer who has been responding positively to identified customer needs and emerging trends in the marketplace in the delivery of innovative and custom-designed food flavorings is Flavor Systems International based in Cincinnati, Ohio, in the United States (Anonymous 2004b). The company has responded to the recent damage to the vanilla bean crop in Madagascar and the resultant skyrocketing prices caused by developing vanilla extract replacers. These replacers are suitable for use in ice-creams, alcoholic beverages, and bakery products. Also, it has developed a beef extract replacer consequent upon the recent ban on Brazilian beef imports to the United States and the fear of the dreaded and contagious Bovine Spongiform Encephalopathy (BSE), the so-called "mad cow disease". Lastly, liquid concentrates having the real dark-roasted flavor of coffee have been produced to satisfy the demand for the taste of freshly brewed premium coffee as opposed to the comparatively "stale" flavor of dry instant coffee products presently available in the market.

Another motivating factor could be the expansion of sales by the extension of existing lines. By line extension, we mean building on an existing product by addition of new flavors or features. This option is probably the most common approach to new product development because the brand is retained. This brand that is retained is a symbol carrying with it complex and important significance to customers. Moreover, the meaning of a brand is built up by dint of hard work over several years through advertising and by the success of the product sheltered under its umbrella.

A ready example of a line extension is the one involving Maltina®, a nonalcoholic malt drink, which was recently carried by its manufacturers, the Nigerian Breweries PLC, Lagos. The line extension involved introducing other exotic fruit flavors. The manufacturer simply repackaged the old product by dubbing it as "classic" and strawberry- and pineapple-flavored new products were introduced under the same brand name. Really, a forward-looking manufacturing outfit should constantly be experimenting with new product ideas so that at very short notice, the right things can be done to either modify an old product or reposition it in line with current trends.

Lastly, a motivating factor could be the need to reposition an existing product. In an overwhelming majority of cases, this repositioning is achieved solely through sloganeering or new advertising, marketing, and/or repackaging, usually without any tinkering with

the science or engineering of the product. By portraying an otherwise old product in a new light or making fresh claims for the product, consumers can indeed see the product in a new light. This can boost the demand for the product, thus translating into increased sales volume and a more favorable "bottom line".

Unwittingly, the last factor has thrown up a pertinent question. Does merely repositioning or repackaging of an otherwise old product make it a new product? Or put the other way round, when is a "new" product really new?

5.1.1 When is a New Product Really New?

A product is considered new if it is new in any way, for the company and/or the consuming public and potential customers (Albrecht 1981). Therefore, if a company starts to manufacture a product that is already marketed by other manufacturers, although the product is old in the market, the product marketed newly is considered new because at least the brand name will be different. Also, if the manufacturer succeeds in convincing the consumers of an otherwise old and unchanged product, about the "newness" of the product by making fresh claims or successfully repositioning the product, then the otherwise old product becomes a new product as far as the consumers are concerned. This newness will be reflected in the product experiencing a new lease of life, so to say, by passing through another life cycle. A few examples from the larger food industry will illustrate these points.

Let us begin with a case of a rather peculiar nature. In the past, cookie manufacturers have successfully changed the perception of their product as a dessert to a snack, solely through advertising and marketing. This was achieved in part by seeking different market targets, through marketing in smaller packages, that is, without changes to the physical product. Thus the initial advertising and promotion blitz, followed by the

TABLE 5.1 Some New Flavors and New Flavored Food Products Introduced into the International Market Since 1990.

Product Name and Description	Flavor/New Feature	Manufacturer
Chelsea Teezers (gin blend)	Ginger and other exotic flavors	Continental Distillers, Ikeja, Nigeria
Vegetarian chicken flavor (used in meatless textured proteins)	Vegetarian flavor	Gold Coast Ingredients, Inc., California, USA
Beef extract replacer	Safeguard against mad cow disease	Flavor Systems Int'l., Inc. Cincinnati, USA
Fanta® pineapple (soda)	Pineapple flavor	Coca Cola Canners of S.A (PTY) Ltd., Wadeville, South Africa
Bien® (bottled water)	Apple flavor	Pharma-Deko PLC, Agbara, Nigeria
Drink fit® yogurt (yogurt drink)	Strawberry flavor	DrinkFit GmbH, Schlüchtem, Germany
Fitnesse™ (toasted flakes of rice, wheat and corn — breakfast cereal)	Malt flavor	Nestlé S.A., Vevey, Switzerland
Coco Pops® (toasted rice — breakfast cereal)	Chocolate and malt flavors	Kellog Marketing & Sales Co., Manchester, England
Sugar Puffs (wheat puffs — breakfast cereal)	Honey and brown sugar flavors	Quaker Trading Ltd., Southhall, England
Chocolate Syrup (Sugar syrup)	Chocolate flavor	Fleming Co. Inc., Oklahoma City, USA
Khao Shong™ Nuts (Roasted peanuts)	Coffee flavor	Lily Industry Co. Ltd., Bangkok, Thailand

increased frequency of use as light meals as opposed to its sole use as the final course of meals, caused the product to go through the early stages of the typical product life cycle. Also, in the marketing of orange juice, through extensive promotion, the same physical product was successfully repositioned as orangeade, diet supplements, salad component, garnishing for meat, fish, and seafood, and many other culinary uses.

Apart from repositioning through advertising and marketing, new products are so usually created through improved formulation and packaging. These classes of new products are so evidently new that further pontification appears unnecessary.

To round off the discussion on the need for new products in the flavor, as well as the larger food industry, a list of some new flavors as well as new flavored food products that have been introduced into the international market in the last couple of years are presented in Table 5.1

5.2 AN OVERVIEW OF LEGISLATIVE GUIDELINES ON NEW PRODUCT DEVELOPMENT

As stated in the introductory comments to this chapter, legislative requirements constitute the most important impediment tending to slow down innovation and new product development in the flavor industry in particular, and in the food and consumer industry in general. It is therefore apposite to give a bird's eye view of these regulatory guidelines, with particular reference to those aspects that have a direct bearing on new product development.

The earliest evidence of the existence of food laws appears to be in ancient Egypt (Luckey 1972). Luckey (1972) also cited an Indian law of 300 BC, which prohibited the adulteration of grains, scents, and drugs. However, the earliest records in modern history of food laws can be traced to England in the thirteenth century and later on, to the United States in the nineteenth century. For instance, as early as 1202, King John of England proclaimed the first English food law, which prohibited the adulteration of bread with other nonwheat flours. In the United States, however, serious attempts at food legislation did not commence until around 1880, when the individual states began to pass laws against food adulteration.

The first significant milestone in the checkered history of food legislation in the United States was the signing into law of the original Food and Drugs Act, by President Theodore Roosevelt, in 1906. This act prohibited misbranding and adulteration of foods, drinks, and drugs. Although the original act has been revised through the addition of new provisions (FDA 1966), the basic provisions have been retained. Further information on milestones in the food and drug law history in the United States can be obtained from the Food and Drugs Administration's (FDA) backgrounder (FDA 2002).

In conclusion, it is pertinent to lay emphasis on two important issues. First, there now exist food laws in virtually all countries of the world, and second, food legislation practices in several of these countries have benefited tremendously from the records of modern food laws in England and particularly, the United States. This is because other countries often emulate such laws that a country, especially a developed one, has used to solve a problem with satisfactory results. Thus the provisions in all these food laws are addressing the same central issues relating to marketing standards of identity, misbranding, adulteration, and so forth. Secondly, it is to be noted that the work of international bodies such as the World Health Organization (WHO), Food and Agriculture

Organization (FAO), and the European Union (EU) is assuming increasing prominence over national food laws as a way of harmonizing the diverse national regulatory laws and guidelines in the interest of international trade. The contributions of the international body established by the FAO and WHO to develop international food standards, that is, The Codex Alimentarius Commission, is worthy of note in this regard.

The following is a brief review of the legislative guidelines on new product development, as well as a review of the work of The Codex Alimentarius Commission.

5.2.1 Legislative Guidelines on Food Product Development

Food laws and standards stipulate the exact specifications for items of commerce. The standards establish an authority for the quantity, weight, value, or quality of specific items. They also concern identity, quality, and fill of container. This is generally with a view to promote honesty and fair dealing between the manufacturers and the consumers. Thus, the laws strictly forbid fraudulent practices, especially as regards food additives. Other prohibited acts include adulteration and misbranding.

For the purpose of most food laws, a simple definition of an additive as "a substance or mixture of substances other than the basic food, present in food during production, processing, packaging and storage" (Luckey 1972) will suffice. In this regard, it is to be noted that additives may either be intentionally added to food or may occur by accident. Thus, preservatives may be intentionally added to enhance the keeping quality of foods. On the other hand, residues of pesticides and herbicides used in agricultural production, when not properly removed from the end products, constitute accidental food additives.

Thus several sections of many food laws have guidelines on tolerance for pesticide chemicals, including insecticides, bactericides, fungicides, and mycotics, where tolerances are proposed . Also, there are guidelines on exemption of such pesticides and other agricultural chemicals from the necessity of a tolerance in certain circumstances. For the purpose of our discussion here, we are going to discuss only intentional food additives that are generally used to enhance either the stability or the attribute components of quality of the product. Also, there will be a brief discussion of regulations on adulteration and misbranding.

5.2.1.1 Guidelines on Food Additives. There are general requirements to be satisfied in law with respect to food additives (FAO/WHO 1956). Usually, these requirements vary from one additive to another. However, generally speaking, these guidelines require the following:

1. There must be a demonstrable need for an additive before it is used. This applies mostly to nutritional additives like iodine and vitamins. For instance, the use of iodine is indicated in the case of a population with a high incidence of goiter.
2. A suitable conveyor or vector for the additive must be specified.
3. The keeping quality of the additive must be guaranteed. In other words, it must be scientifically proven that the additive is stable and will not react with the vector.
4. The additive must not impart any undesirable flavor, odor, color, or texture to the food. That is, it must not make the food unacceptable in any way.
5. The additive must be able to retain its serving properties.
6. The safety of the additive must be indicated and ensured.
7. The additive must not be antagonistic to the food. For instance, the additive must not bind to any of the nutrients or make them unavailable to the body.

Furthermore, a processor that intends to use an additive must file a petition with an appropriate authority to establish its safety (FDA 1966). In the petition, the following details must be specified:

1. Name, chemical identity, structure, and composition of the compound.
2. All chemical properties of the compound, including the intermediate compounds that may be formed from the compound. This is because the intermediate compounds may be toxic, although the main compound may not be.
3. Minimum and maximum levels of use. This has to do with the cumulative effect of the additive.
4. Details of toxicological studies and other scientific experiments undertaken to establish the safety of the additive.
5. The methodology of these experiments, which must relate to the proportions of the additive to be used in the food vector.
6. The experimental materials and methods and especially the age of the experimental animals, which must reflect the age group of people for which the additive is intended.
7. Recommended duration of use of the additive.

Suffice it to say that the required details, which must be filed with the appropriate regulatory authority, have been derived largely from the FAO/WHO prescribed guidelines for the international testing of food additives to establish their safety (FAO/WHO 1958). The foregoing notwithstanding, it needs to be pointed out that the prescribed guidelines, being a scientific document, is more detailed than the legislative version of the safety requirements. This is more so especially with regard to the conduct of the toxicological studies. For instance, the FAO/WHO prescriptions require that the design of the experiments be furnished. Also there are guidelines on the number and selection of animal species.

In conclusion, it needs to be pointed out that the successful use of experimental animals depends on careful selection and maintenance of the animals. Secondly, investigation of toxicity should not involve inadequate diets. Lastly, to limit the number of experimental animals, reliance must be based on the ability to observe responses in the majority of animals receiving doses far in excess of the recommended levels for human consumption.

5.2.1.2 Regulations on Adulteration and Misbranding. All food laws forbid fraudulent practices as regards food as an article of trade. Such laws cover the basic offences of adulteration and misbranding. A food is considered adulterated under the following circumstances:

1. If the prepared food contains injurious substances. Such injurious substances may be naturally present, added, or present in the container.
2. If the food has been prepared, packed, or held under insanitary conditions.
3. If the food contain parts of a diseased animal.
4. If it is radiated beyond the prescribed dose.
5. If part of it is omitted for instance in the case of omission of the more expensive part of fabricated foods.
6. If part of the food has been substituted in a fraudulent way.

7. If part of the food is damaged or contains some inferiority, or the inferiority is concealed in same way.
8. If an untypical material has been added to increase bulk.

On the other hand, a food product is deemed misbranded under the following conditions:

1. If it carries a false label or is falsely labeled. A false label refers to the label of a different product and false labeling refers to wrong or false claims on the label.
2. If the required statements are not stipulated. For example, failure to declare that the food contains in part or whole genetically modified organisms (GMOs).
3. If a food product is an imitation and the imitation was done in a fraudulent way.
4. When the standard of quality or fill of the container is tampered with.

5.2.1.3 Labeling of Genetically Modified Foods. In the brief discussion of food regulations in relation to misbranding, only a tepid reference was made to labeling with regard to genetically modified (GM) foods and food ingredients. However, in view of the potential risks involved in their use, and the escalating debate about their safety and mandatory labeling of GM foods (Smyth and Phillips 2003), there is a need to make some clarifications.

First and foremost, it is to be noted that several regulatory bodies have ruled that there is no scientific evidence to suggest that GM foods involve any new or magnified risks. Despite these assurances, many civil society groups and a large portion of consumers are still not convinced about the safety of these foods. Secondly, due to the public concern about the long-term safety of GM foods and food ingredients, the Labeling Committee of the Codex Alimentarius Commission has been meeting for the past ten years in an effort to develop international standards for labeling of GM foods. No definitive guidelines have yet been issued. Thirdly, some national regulatory agencies have issued tentative enactments demanding mandatory labeling of all GM foods and ingredients in the absence of international guidance. Fourth, even in countries that have made these enactments, there is a raging controversy on the appropriate structure of such labeling. Lastly, some food manufacturers, without any compulsion, have taken the initiative to indicate on their labels whether or not their products contain GM foods and ingredients. The last has surely not been heard on the labeling of GM foods.

5.2.2 The Codex Alimentarius Commission

As was pointed out earlier, the work of international bodies such as the World Health Organization (WHO), and the Food and Agriculture Organization (FAO) have assumed prominence over national food laws as a way of harmonizing the diverse national regulatory laws and guidelines in the interest of international trade.

Going down the memory lane, both the FAO and WHO were established in 1945 as specialized agencies within the United Nations (UN) system. The FAO had the mandate to help member governments increase food resources and improve the distribution of food and agricultural products, thereby improving the nutritional level of their people. The WHO on the other hand was mandated to deal with all matters concerning health and general well-being, including nutrition. Because of the linkages and interpenetrations

between food, nutrition, and health, the FAO and WHO have had to form joint expert committees to handle cross-border issues.

The first of such bodies was the WHO/FAO Joint Expert Committee on Food Additives (JECFA), which was formed in 1955. Another of such bodies was the FAO/WHO Codex Alimentarius Commission (CAC), which was formed in 1962. The CAC, with its secretariat at the headquarters of the FAO in Rome, is the main international body concerned with the setting of international food standards. Further information on the statutes, rules, procedures, and membership of the CAC can be obtained from its procedural manual (CAC 1997), and information on the current status of the CAC can be obtained from Jukes (1999).

5.3 FLAVOR CREATION AND NEW PRODUCT DEVELOPMENT

The creation and development of flavorings in food product development cuts across several disciplines. The practitioners are the flavor chemists, flavorists, food technologists, and marketers. For specialized flavors, some specialists, usually within the main groups, may be involved. Generally, a synthetic chemist may be required in addition to the flavor chemist. Also, in addition to the food technologists, nutritionists, dietitians, chefs, home economists, and packaging engineers may be required. The point to be emphasized is that the simplified discussion here is just to illustrate the general principles involved in flavor creation. Nowadays, specialized companies who offer them for sale to food manufacturers develop the flavorings. We can then partition the various tasks involved in flavor creation between the marketer, food techologist, flavorist, and flavor chemist. Figure 5.1 shows the typical roles played by the four professionals in the creation of flavorings in food product development. Let us now examine each of these tasks in greater details.

5.3.1 Generation of New Product Ideas

New product ideas may emanate from the marketing team in the course of their interaction with the public. On the other hand, the idea may emanate from the report of the food technologist on a new flavor from a natural product. It may even emanate from the management. Whichever way the new product ideas are generated, the flavor chemist takes over by isolating, identifying, and synthesizing some of the identified components of the natural flavors to be imitated, when available synthetic compounds are inadequate. The flavorist generally establishes the flavor profile, and then compounds the imitation flavor from synthetic compounds. These and other subsequent roles will now be discussed.

5.3.2 Isolation, Identification, and Synthesis of Flavor Compounds

The task of isolation, identification, and synthesis of flavor compounds in food odor research will be broken down into three stages:

1. Isolation and concentration of volatiles;
2. Identification of compounds; and
3. Synthesis of flavor compounds.

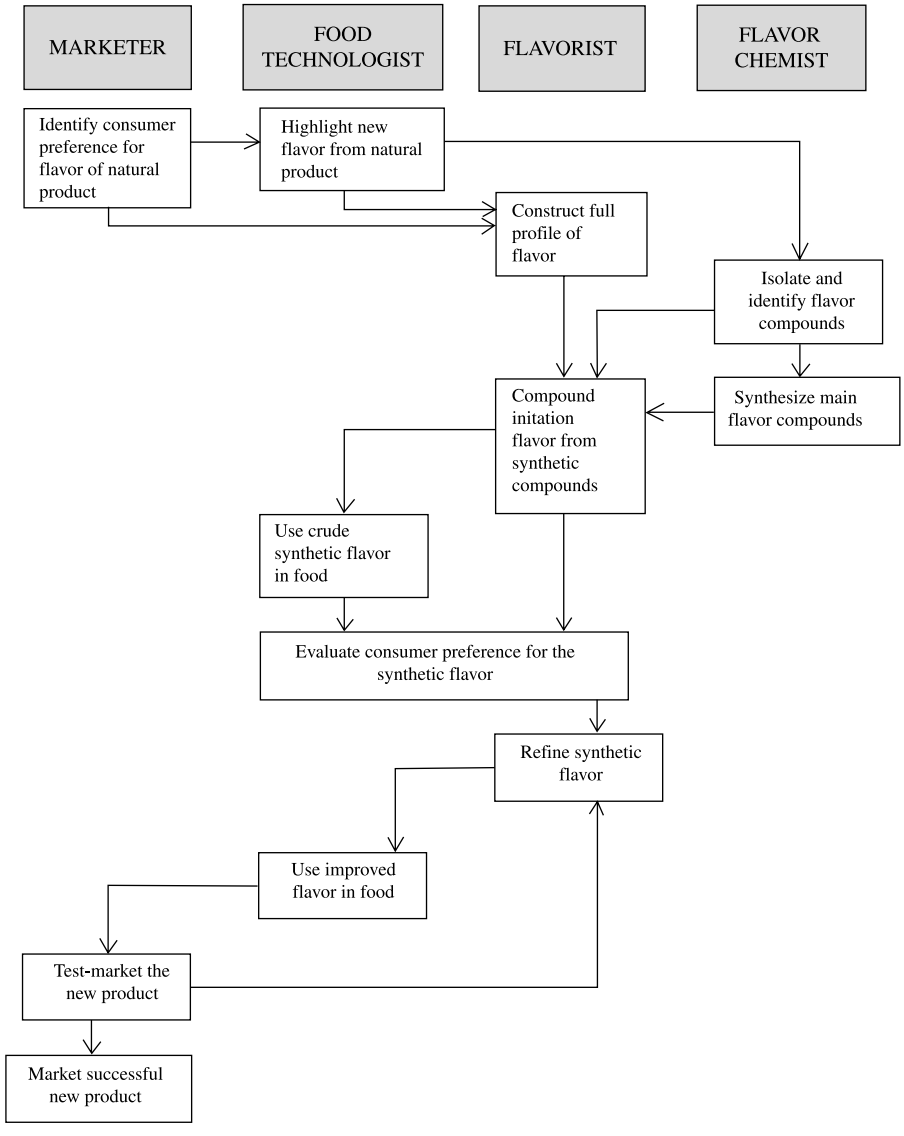

Figure 5.1 *Roles of the marketer, food technologist, flavorist and flavor chemist.*

5.3.2.1 Isolation and Concentration of Volatiles. It must be realized that there are two basic approaches in food odor research. The first involves carrying out direct vapor analysis or headspace vapor analysis. The other approach is to carry out total volatile analysis. Each of the two approaches has its own merits and demerits, which have been discussed by Weurman (1969). The first approach is more straightforward, because the volatiles are already in vapor form and can readily be collected and injected into analytical equipment. So, in effect, the isolation and concentration procedures apply more to the total volatile analysis.

TABLE 5.2 Location of Principal Flavor Compounds in Selected Food and Allied Food Products.

Food and Allied Products	Location of Principal Flavor Compounds
Poultry	Meat (muscle) plus fat under the skin
Fish	Fish oils and fats
Milk and dairy	Milk fat
Potato	Potato fat
Hops	Oleoresin

The process of isolation of the natural flavor compounds entails two steps: (1) Separation of compounds from the original material, and (2) concentration of separated compounds. In order to isolate the volatiles, it is very important to know where flavor compounds reside or, put another way, where they abound in high concentrations in the natural material, before they can be successfully separated. Table 5.2 shows the locations of principal flavor compounds in selected agricultural materials. Generally, when in doubt or when dealing with a new natural product, the fats and oils, that is, the lipid fraction of the material, should be the first suspect.

The first steps in the separation of flavor compounds from the native material generally entail one form of size reduction or another. By this, we mean unit operations such as cutting, grinding, mincing, pressing, or centrifuging. This first step is followed by distillation or solvent extraction, depending on the physicochemical properties of the target compounds. As virtually all flavor or odoriferous substances are volatile in nature, almost invariably the process of choice is distillation.

In this first distillation process where a crude extract is expected, the regular single-plate, total take-off distillation system is often preferred. The other distillation procedures have been reviewed by Weurman (1969) and Parliament (1997). Right from the distillation stage, special precautions are to be taken. For instance, it is essential to work at subzero temperatures under nitrogen, as this prevents chemical and enzymatic side reactions. Following the successful removal of the compounds from the native material, some form of concentration needs to be carried out. The concentration step becomes necessary because very dilute solutions are obtained from the first-step isolation. Concentration of the compounds can be achieved by any of the following methods: (1) Freeze drying; (2) adsorption; (3) extraction; (4) fractional distillation or stripping; and (5) derivative formation. These concentration techniques have been extensively reviewed by Weurman (1969).

5.3.2.2 Identification of Compounds. Identification of the flavor compounds in the concentrates start with their detection using chromatographic techniques. Almost invariably the identification is done by combined gas chromatography/mass spectrometry (GC/MS). Usually, the GC retention times and mass spectra of the volatile compounds are compared with those of reference standards (Garruti and others 2003). Further, the respective structures of the compounds can be confirmed through nuclear magnetic resonance (NMR) and infra red (IR) spectroscopy (Werkhoff, and others 1998). An NMR system is shown in Figure 5.2, and an IR spectrophotometer in Figure 5.3.

5.3.2.3 Synthesis of Flavor Compounds. Usually, the flavorist possesses an intimate knowledge of the aromatic profiles of hundreds of natural and synthetic flavoring components. Thus, following isolation and identification of the natural flavor compounds,

Figure 5.2 *An NMR system.*

there are several hundreds of synthetic compounds available to the flavorist from which the natural flavor compounds can be compounded or recreated synthetically. Both the knowledge and the artistic skill of the flavorist come in handy in performing this delicate task. However, realizing the complex nature of natural flavorings in terms of the large array of volatiles responsible for the flavor of a single natural product and the overbearing influences of those compounds present in seemingly negligible fractions, organic synthesis of some identified flavor compounds with no ready synthetic equivalents become inevitable.

Figure 5.3 *An IR spectrophotometer.*

Organic synthesis of flavor volatiles is the exclusive preserve of the synthetic/flavor chemist. It is a specialized area requiring a lot of skill. The complexities of organic synthesis notwithstanding, there are several excellent works on the syntheses of several components of natural flavorings (Finato and others 1992; Sanders and Seidel 1992; Schmidt and others 1992; De Kimpe and Keppens 1996).

5.3.3 Formulation and Compounding of Synthetic Flavorings

The flavorist executes the formulation and compounding of the synthetic flavorings. In this task, the flavorist is not only adept at, but is in fact first among equals, in the sensory evaluation of flavor and odoriferous compounds. The work of the flavorist in this regard is accomplished in two stages: (1) Characterization or sensory analysis of the flavorings, and (2) compounding of the imitation flavoring.

5.3.3.1 Sensory Analysis of Flavorings. Flavor creation is an iterative process of trial and error. Oftentimes, it necessitates constant assessment of odor and flavor profiles. First, the new flavor from the natural product has to be characterized. Second, several experimental blends of the synthetic flavor have to be characterized progressively and compared with the natural flavor. These comparisons often reflect the direction for improvement or refinement of the crude synthetic flavor. Usually a combination of artistic and scientific tools is employed in the task of sensory evaluation of flavorings and flavor compounds. This task is commonly accomplished through (1) flavor profile analysis, (2) quantitative descriptive analysis, and (3) the spectrum method (Bett and Grimm 1994). All the methods involve teamwork, usually among highly trained panelists or judges. These judges are well experienced in the evaluation of flavor and flavor compounds, especially by sniff testing.

Construction of Flavor Profile. Flavor profile analysis is a way of completely describing all the flavor components of a material. In this method, there is a complete description and recording of the intensity, order, and amplitude of each of the flavor compounds or notes. Thus the product's aroma, flavor, and aftertaste are recorded on a simple universal scale. Normally, about four to six trained judges sniff the material to be evaluated and then they discuss in order to harmonize their judgments and come to a consensus in regard to each character note or descriptor. After reaching a consensus, then the threshold of the flavor compound is recorded on a scale. The scale usually consists of the following: (1) not present or below threshold, (2) threshold, (3) slight, (4) moderate, or (5) strong (Bett and Grimm 1994).

In an overwhelming majority of cases, a detailed flavor profile of a natural flavoring to be imitated has to be constructed prior to the compounding of the synthetic imitation. Also, after the synthetic imitation is compounded, the profile of the synthetic flavor has to be constructed in order to compare how close the imitation or synthetic flavor is to the natural flavor.

Quantitative Descriptive Analysis. In this technique, trained judges are asked to quantify, in order of occurrence, the sensory quality characteristics of a food product. The data obtained from the individuals are used to construct a multidimensional model, which is readily understood in both marketing and research and development environments. A typical model consists of a number of lines corresponding to the flavor

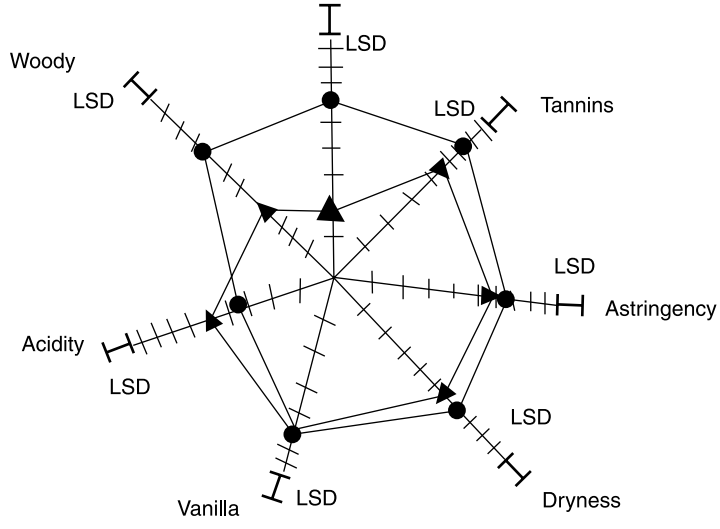

Figure 5.4 *Representation of the flavor of two hypothetical wine samples.*

characteristic to be described. Then the intensity of each flavor note is described on each line with appropriately sized intensity scale. Perez-Preito and others (2003) used this method recently to assess the effect of wood origin, barrel age, and barrel size on the flavor and some other sensory characteristics of red wine. A typical pictorial representation of the flavor of two hypothetical wine samples is presented in Figure 5.4.

This method is different from the previous one in some respects. Although the flavor descriptors are arrived at through a consensus of opinion as in the previous method, that is where the consensus building ends. The individual judges do the scoring of the flavor intensities. Thus, multiple evaluations on each sample are obtained. The multiple scores are then subjected to statistical analysis to improve on the accuracy as well as reliability of the results.

Spectrum Method. The spectrum method separates the overall flavor impression into descriptors just as in the two previous methods. Also, a panel of trained judges derives the descriptors. The judges ensure that the descriptors describe succinctly the flavor notes for which specific standards or references are provided, thus minimizing to the barest minimum the conflict between the descriptors and evaluation of the flavor intensities. Specifically, reference-point intensities are calibrated against specific flavors in commercially available foods, thus reducing the error due to the judges. Therefore, the data can be compared across time and between products, because the effects are due solely to the treatments.

5.3.3.2 Compounding of Imitation Flavoring. The formulation and compounding of the synthetic flavorings is the exclusive preserve of the flavorist. Traditionally, the flavorist creates imitation flavors by using chemicals whose odor and flavor are reminiscent of the natural flavor to be imitated. This is purely an art, which is done by a process of mental association. This task is facilitated by an intimate knowledge of the aromatic

profiles of hundreds of natural and synthetic flavor compounds. The flavorist therefore has hundreds of synthetic volatiles at his/her fingertips, from which to select several permutations and combinations of small groups of synthetic compounds to simulate the natural flavor.

The synthetic flavor need not comprise the wide array of volatile compounds usually associated with natural flavors. The identities of the volatiles need not even be identical with those of the natural flavor, but the formulation must be capable of sensorial simulation of the natural flavor. Further, the flavorist must ensure that the synthetic flavoring will be stable during processing such that it has an acceptable strength in the end product despite the adverse processing conditions.

5.3.4 Trial Use of Synthetic Flavorings

Usually, several experimental blends of the synthetic flavor are produced in the course of the compounding and refinement of the synthetic flavor. At convenient intervals, the food technologist is routinely called upon to creatively use the progressively refined synthetic flavor in selected food vectors so that a preliminary estimation of the consumer preference for the newly created flavoring may be obtained. The food technologist is also well trained to employ different sensory evaluation techniques and appropriate statistical tools to (1) test if there are statistically significant differences between the synthetic and natural flavors; (2) test if one of the flavors is significantly preferred to the other(s); (3) quantify the extent of differences and/or preferences between the test products; and (4) obtain an initial estimate of consumer acceptability of the new flavors. These evaluations often reflect the direction for improvement or refinement of the synthetic flavor under development.

At the final stages of the development, the marketer may be called upon to test-market the product incorporating the newly created flavoring. Marketers are able to obtain an estimate of the consumer reaction to the product through the use of questionnaires and also through interviews.

5.3.5 Marketing of the New Product

Finally, the marketer markets the new product. The new product in this case may either be a newly created flavoring or the new food product in which the newly created flavoring is employed.

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6

Extraction Modes

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6.1 Introduction 148
6.2 Extraction Techniques 148
6.2.1 Enfleurage 148
6.2.2 Extraction with Hot Fat (Maceration) 149
6.2.3 Stripping Using Water Vapor 149
6.2.4 Distillation 149
6.2.5 Extraction with Organic Solvents 149
6.2.6 Extraction with Supercritical Carbon Dioxide 150
6.2.7 Cold Pressing 152
6.2.8 Solid-Phase Micro Extraction 152
6.3 Enhancement Techniques 152
6.4 Extracts Treatment 153
6.5 General Comments 153
6.6 Final Remarks 155
References 155

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

6.1 INTRODUCTION

Extraction is a process of great importance for flavor compounds, because it enables the separation or concentration of a variety of substances from natural feedstock. Presently, the amount of solvent used by industries in conventional extractive processes is estimated as about one million tons per year. The development of extraction techniques can reduce the amount of solvent used and also improve product quality. Thus, there is space, not only to update conventional techniques, but also to take into account the commercial application of new techniques that are studied and used by research groups.

The majority of essential oils are obtained by distillation. This subject, as well as drying methods, will be discussed in Chapter 7. This chapter will focus on other techniques for flavor extraction.

6.2 EXTRACTION TECHNIQUES

Extraction techniques vary according to where the flavor compound is located in a plant. Simões and Spitzer (2003) have cited various methods of extraction, treatment, and conservation applied in the recovery of essential oils and flavor compounds. The most common are presented here.

6.2.1 Enfleurage

This method is only used by perfumery industries, when plants have a small amount of oil with a high commercial value. It is used to extract volatile oil from flower petals. The principle of the method is simple. Every jasmine and tuberose flower resembles a small factory, continually emitting minute quantities of perfume. Fat possesses a high absorption power and, if in contact with fragrant flowers, readily absorbs the perfume emitted. This principle, methodically applied on a large scale, constitutes enfleurage. Petals are placed on a fat layer at room temperature for a period of time. The physiological activities of these flowers continue for 24 h or longer after harvesting. During this period, the fat absorbs the perfume emitted by these flowers. Exhausted petals are exchanged with new ones. Later, the saturated fat is treated with alcohol. The volatile oil is then obtained by distillation of the alcoholic mixture at low temperatures.

In the Grasse region of Southern France, flowers had been processed by this method for a long time until the modern method of extraction with volatile solvents was introduced. In the early days of perfumery, flower scents were extracted with fats and the alcoholic washings of the perfumed fats represented the so-called floral extracts. Despite the introduction of the modern extraction process with volatile solvents, the old-fashioned method of enfleurage, as passed on from father to son through generations, still plays an important role.

Enfleurage on a large scale is today carried out only in the Grasse region, with the possible exception of isolated places in India where the process remains primitive. Every enfleurage building is equipped with thousands of chassis, which serve as vehicles for holding the fat during the process. A chassis consists of a rectangular wooden frame about 2 in high, 20 in long, and 16 in wide. The frame holds a glass plate where the fat is applied at the beginning of the process.

6.2.2 Extraction with Hot Fat (Maceration)

Before the modern process of extraction with volatile solvents was introduced, the perfume of roses, orange blossoms, violets, acacia, mimosa, and others flowers in which the physiological activity stops after picking had been obtained by treating the flowers with hot fat. The principle of this process involves immersion of the flowers in hot fat. In sequence, the same batch of hot fat is placed in direct contact with several batches of fresh flowers, until the fat becomes saturated with the flower perfume. The fragrant fat is then washed with alcohol, as the extract obtained from cold extraction in enfleurage.

The process of maceration is analogous to that of enfleurage, with two fundamental differences. In the case of maceration, hot fat is employed, and the time of contact between fat and fresh flowers is much smaller. Fifty years ago, orange blossoms, if not distilled, were treated by maceration; acacia blossoms, which do not lend themselves to steam distillation, had to be processed exclusively by maceration. Today, the process of maceration is seldom employed. Its products, especially those from orange blossoms, find application only in a few old-fashioned perfume formulas.

6.2.3 Stripping Using Water Vapor

Volatile oils have vapor pressures higher than water; therefore, they can be stripped by water vapor. On a small scale, a Clevenger apparatus, a traditional apparatus used in hydrodistillation, can be used. The distilled volatile oil, after separation from water, must be dried using anhydrous sodium sulfate. In this classical procedure, the formation of new compounds via degradation is possible, because of the high temperatures used during the stripping or water separation stages. This technique is preferentially used to extract oils from fresh plants in flavor extraction.

Using batch or continuous operation, this process can be used in the deodorization of vegetable oils on a large scale, in which the stripping is accomplished using water vapor under vacuum conditions and elevated temperatures (Heldman and Hartel 1999). The objective is the removal of volatile fatty acids from the oil; therefore, the vapor stream is not the main product as in flavor extraction from plants.

6.2.4 Distillation

Distillation is widely used in the chemical processing industry for the separation of components with different volatilities. It is mainly used for the separation of volatile components, like flavors, in the food and cosmetic industries. Hydrodistillation (steam distillation) is a common type of distillation in flavor extraction. Its main feature is that the matrix containing the substances to be extracted must be immersed in boiling water. Distillation processes are described in more detail in Chapter 7.

6.2.5 Extraction with Organic Solvents

Robiquet first applied the extraction with organic solvents to flowers in 1835. Buchner, in 1836, and Favrot, in 1838, investigated the use of diethyl ether as a solvent. During the nineteenth century, several solvents were applied and patents pertaining to the process and its main extraction products were registered in several countries of Europe. Gradually,

the method attracted the attention of the manufacturers, and industrial workers conducted large-scale experiments independently. Finally, some processors were forced to adopt the volatile solvents, and special extraction plants were constructed in addition to their existing enfleurage buildings.

Volatile oils are extracted preferentially with nonpolar solvents, such as ether, petroleum ether, or dichloromethane. However, these solvents extract other lipophylic compounds besides the volatile oils, and these compounds decrease the commercial value of the oils. The undesired extraction of other compounds is difficult to prevent.

The principle of extraction is simple. Components present in a solid raw material are extracted by dissolving them in a liquid solvent. This process is called leaching or solid–liquid extraction. The raw material is placed in specially constructed extractors where its contact with carefully purified solvent, usually petroleum ether, is promoted at room temperature. The solvent penetrates the raw material and dissolves the natural flavor components along with some waxes and albuminous and coloring matter. The solution obtained is removed from the extractor and pumped into an evaporator to be concentrated at a temperature that is as low as possible. After the solvent is completely removed, a concentrated flavor is obtained. Temperatures applied along the entire process are lower than those used in distillation. Compared with distilled oils, the essential oil obtained by solvent extraction, generally, has a flavor that better represents the original flavor present in the raw materials.

Despite its advantages, solvent extraction cannot entirely replace steam distillation, which remains the principal method for the isolation of essential oils. Steam distillation can be carried out in remote and primitive parts of the world, whereas solvent extraction needs complicated and expensive apparatus and well-trained workers. In solvent extraction, running expenses are comparatively high. Therefore, a mistake in the operation can be costly. Extraction with solvents can be applied advantageously to higher priced materials, like the extraction of essential oils present in flowers. The products of solvent extraction possess one main advantage: their true-to-nature odor. In addition, certain types of flowers, such as jasmine, acacia, mimosa, and violet, do not yield their volatile oil in steam distillation.

Two factors must be investigated and carefully defined to guarantee the success of solvent extraction: the selection of the solvent and the extraction unit. Currently, the solvent selection must be made in accordance with legislation governing the use of the extract (food, cosmetics, or perfumery), as well as with client specifications, which can be more restrictive than the legislation itself. The selected solvent influences the composition of the extract (different solubility parameters), its sensory quality, and the extraction yield (Danisco 2001).

6.2.6 Extraction with Supercritical Carbon Dioxide

This technique allows the recovery of different kinds of natural flavors and volatile oils with high efficiency. If the costs and technical problems involving high-pressure operation could be solved, it would be the preferred method to be applied in the industrial extraction of volatile oils. No trace of solvent remains in the final product, which represents a higher degree of purity when compared with those obtained by other techniques.

In the extraction, the solvent, carbon dioxide, is pressurized to at least 73 bar (critical pressure) at a temperature higher than 31°C (critical temperature). Above these critical values, the carbon dioxide reaches the "supercritical state". In this state, the viscosity is

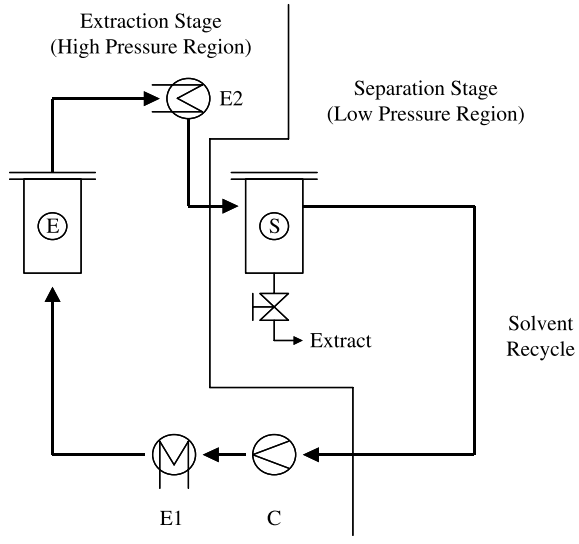

Figure 6.1 *Typical supercritical extraction process.*

similar to that of a gas, facilitating its penetration into the solid matrix, and its capacity of dissolution is similar to that of a liquid. After extraction, the solvent is separated from the extract by lowering its pressure, causing the carbon dioxide to convert to the gaseous phase and to lose its high dissolution capacity. The product can be completely separated from the solvent, which is again compressed and recycled into the process.

Solvent extraction from solids typically consists of two main stages: extraction and separation. A schematic description of a typical supercritical extraction process can be seen in Figure 6.1. In the extraction stage, the supercritical solvent flows through a fixed bed of solid particles and dissolves the extractable components. The direction of the solvent flow through the fixed bed can be upward or downward. At high solvent ratios (ratio of supercritical solvent flow to the amount of solid), the influence of gravity is negligible. The geometry of the fixed bed should also be considered an important parameter in the process design.

The temperature and the pressure inside the extraction unit define the relevant physical properties of the carbon dioxide (namely density, viscosity, and mass diffusivity) that change significantly with small changes in temperature and pressure near the critical point. This behavior causes changes in the solvent dissolving capacity, which makes it possible to define a specific set of compounds to be extracted. On the other hand, it makes it difficult to control the extraction unit operationally. The typical values of these operational parameters are 1.01 to 1.10 for reduced temperature (ratio between operational and critical temperature) and 1.01 to 1.50 for reduced pressure (ratio between operational and critical pressure).

The solid material will be depleted from the extractable material in the direction of the flow. Therefore, the concentration of the extracted components increases in the direction of the flow in the supercritical solvent and in the solid material. This behavior is a function of the operational conditions, of the kinetic extraction properties in the solid matrix, and of the solvent power of the supercritical solvent (Brunner 1994).

In the separation stage, the solution that exits from the extraction unit is expanded in a low-pressure vessel. The carbon dioxide vaporizes and the extract is collected by gravity or by entrainment through a porous media.

Supercritical fluid extraction using carbon dioxide has found commercial application in decaffeination of coffee beans, extraction of essential oils, removal of coloring agents, and concentration/purification of flavor components (Moyler 1993; Hawthorne and others 1993; Ayala and Luque de Castro 2001; Bernardo-Gil and others 2003).

Some solvents, such as water (critical temperature and pressure 374°C and 220 bar, respectively), have a higher critical pressure and temperature then carbon dioxide. Thus, extraction in a supercritical condition would require more severe conditions, which would result in higher costs and technological difficulties. There is also the possibility of degradation of thermolabile substances. Therefore, it is usual to adopt operational conditions below the solvent critical point, even with carbon dioxide. An example is the continuous subcritical water extraction process (CSWE), tested by Jiménez-Carmona and others (1999) in the recovering of flavor compounds from marjoram essential oils.

6.2.7 Cold Pressing

During cold pressing, the fluids entrained inside the raw material are removed by expression. This method is used to extract volatile oils from citric fruits. The pericarp of the fruits are pressed and the fluid, which contains the volatile oil, is separated. Water can be used to enhance oil removal from the solid residue. The oil is separated from the emulsion by gravity, centrifugation, or fractionated distillation.

6.2.8 Solid-Phase Micro Extraction

Solid-phase micro extraction (SPME) is a technique that started in the 1990s with applications not yet consolidated, even from a theoretical point of view. The initial studies were on laboratory applications, exploiting fundamental aspects of the method.

SPME is a process involving a multiphase equilibrium. It is a micro technique, in which the process of extraction of the analytes takes place at an unusual scale. The basic device consists of a thin slapstick of optical fiber of fused silica with 10 mm of one of its ends recovered with a thin film of polymer. SPME consists of two basic steps: partition of the analytes between the fiber and the sample (matrix), and desorption of the concentrated extract in an analytical instrument (gas chromatograph). The sample consists of an aqueous solution with solid particles in suspension that has several interactions of adsorption with the analytes besides the gas phase. The desorption time depends on several parameters, the most important of which are the volatility of the chemical compounds, the kind of injector, and the temperature (Zhu and others 1999; Cai and others 2001; Tellez and others 2004). The great advantage of the method is the extraction with the direct quantification of flavor compounds present in different matrices – gas, liquid and solid.

6.3 ENHANCEMENT TECHNIQUES

A procedure that can be used to increase the extraction potential is the previous freezing of vegetable raw material. Freezing causes rupture of the cell wall, facilitating the action of the solvent on the products of cellular metabolism. Moreover, conventional extraction

methods can have their yields enhanced by ultrasonic waves, which improve the interaction between the solvent and the analyte molecules.

Extraction using microwaves is a process in which the solvent, in contact with the raw material, is heated by radiation in the microwave range to improve the dissolution of desired substances from the natural matrix into a solvent. Since 1985, application of microwave heating in extraction processes from solid matrices has demonstrated good efficiency. Solvent extraction enhanced by microwave radiation has been used on a large scale to obtain essential oils from various vegetable raw materials. The advantages are its simplicity and the short period of extraction. The first publications about extraction using solvent and microwave involved yeast, hop, broad beans, corn, and chestnut. Solvents like methanol and a mixture of hexane and water have been used, and the extraction yields obtained using microwave are much better than without it.

Ganzler and Salgo (1990) described the use of microwave to improve the extraction of organic substances from solid matrices such as soil, seeds, and food. These researchers used a conventional microwave oven to irradiate the solid matrix with the solvent seven times, for 30 s each time. The extraction assisted by microwave was more efficient for polar substances than extraction using a Soxhlet apparatus.

Comparative measurements were performed using a Soxhlet extractor (conventional extraction technique) and extractions assisted by sonic waves and microwave. The extraction with microwave was a more feasible alternative than the Soxhlet technique or the use of sonic waves. Furthermore, it used a smaller amount of organic solvent, reduced extraction time to less than 10 min, and allowed extraction of several samples simultaneously (Lacerda 1999).

6.4 EXTRACTS TREATMENT

Often it is necessary to clarify, to neutralize, or to rectify the extracted volatile oils. Rectification, drying, or low-pressure water vapor jet utilization allows the extraction of irritant or unpleasant flavor compounds, leading to final products with higher aggregated value. Deterpenation, which is a special kind of rectification, aims at the reduction of monoterpenes in the oil. The use of chromatographic techniques, in particular exclusion chromatography, allows the separation of the volatile oils from other lipophylic nonvolatile compounds. A partial fractionation allows the separation of the original solution into a fraction rich in monoterpenes and another rich in sesquiterpenes.

6.5 GENERAL COMMENTS

Extraction processes can be applied to selectively obtain a more specific and standardized product, with a higher degree of purity. Extraction of volatile substances is still performed using adequate solvents and applying different physical processes. The disadvantages are the possibility of chemical alteration of the substances, the degradation of thermolabile compounds, and the occurrence of undesired reactions caused by an eventual need for higher extraction temperatures to obtain better yields. After extraction, solvent separation from the final product is another point of difficulty. For example, when using hydrodistillation, besides heating, there is the inconvenience of direct contact of the material with hot water, which can cause hydrolysis of some substances. Extracts obtained from organic solvent extraction often contain a solvent residue that could be seen, in many situations,

as a contamination that must comply with a maximum level defined by legislation. The lower the limit, the more difficult and more expensive the process of solvent separation.

Due to differences among separation methods, it is common to find in the literature studies comparing the performance of new methods with the traditional ones, mainly hydrodistillation. Foods, essential oils, and medicinal plants are raw materials often used in these studies. Some of these results are described below.

Ondarza and Sanchez (1990) compared steam distillation (hydrodistillation) and supercritical fluid extraction for the isolation, separation, and purification of some compounds from spices. The use of supercritical CO_2 extraction presented a commercial advantage because of the fact that the oils obtained were constituted mainly of oxygenated hydrocarbons and had a low content of monoterpenes that could mask the natural flavor of the essential oil.

Blanch and others (1993a,b) investigated factors affecting the simultaneous steam distillation and solvent extraction of volatile components from foods using solvents with many densities. A new apparatus was developed in order to minimize losses of the most volatile compounds. The cooling surface was enlarged by the introduction of a cooled jacket at the end of the condenser. The jacket acted as a second cooling system.

Jiménez-Carmona and others (1999) compared continuous subcritical water extraction (CSWE) with hydrodistillation in the recovery of flavor compounds from marjoram essential oil. CSWE was faster (15 min compared to 3 h) than hydrodistillation, and provided a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes). CSWE allowed substantial cost savings in terms of both energy and raw material. Its extraction efficiency was about five times higher than that of hydrodistillation.

Gámiz-Garcia and Luque de Castro (2000) reported the use of CSWE in the extraction of essential oils from a medicinal plant and compared its performance with hydrodistillation. The advantages observed were shorter extraction time (50 min instead of 4 h for hydrodistillation), lower energy cost than for hydrodistillation, and the possibility of controlling the composition of the oil by changing the extraction parameters (temperature, fluid flow rate, and static extraction time). Another advantage of CSWE was that the design of automated and controlled plants is more feasible.

Sarrazin and others (2000) compared five different extraction methods to determine which provided the most representative coffee flavor extract, including supercritical extraction with CO_2, extractive distillation, oil recovery under pressure, and vacuum steam-stripping with water or organic solvent. The vacuum steam-stripping with water provided the most representative flavor extraction, followed by stripping with methylene chloride. About two-thirds of the flavor was recovered in solvent extraction, and approximately 5% was lost in the concentration step.

Padukka and others (2000) studied five different extraction methods, among them hydrodistillation, solvent extraction using hexane, diethyl ether and chloroform, and supercritical CO_2 extraction, to obtain lemon oil from a complex of cyclodextrin and lemon oil. Volatile flavor components were successfully extracted by hydrodistillation under carefully controlled conditions. All three solvents tested could be used; however, hexane was more efficient and easier to handle, with the organic phase readily separated. Chloroform was less efficient in terms of phase separation. Supercritical fluid extraction was not successful and extracted only 33% of the total encapsulated oil after 7 h at 45°C. The profiles of the flavor compounds of the oil extracted by the different solvents were similar.

Kondo and others (2002) studied the supercritical fluid extraction of cold-pressed lemon oil carried out in semibatch and continuous countercurrent modes. Continuous operation at a linear temperature gradient from 313 to 333 K at 8.8 MPa showed the highest selectivity, which increased with an increase in the solvent-to-feed ratio. Operation with a linear temperature gradient allowed a more selective separation of oxygenated compounds than that obtained with a uniform temperature along the column.

Diaz-Maroto and others (2002) compared hydrodistillation and solvent extraction with supercritical CO_2 to recuperate the volatiles from spices (oregano, basil, and mint). The extracts obtained using the two methods were very similar in composition. Supercritical CO_2 extraction required less extraction time, did not allow thermal degradation and solvent contamination of the samples, and preserved the natural character of the fresh product.

6.6 FINAL REMARKS

After analyzing the available methods to extract and to concentrate flavor compounds, some final remarks can be made.

- Hydrodistillation: Not all oils can be processed by hydrodistillation, because boiling water and steam can have a deteriorating influence upon delicate flavor substances. Moreover, certain raw materials yield no oil during hydrodistillation.
- Enfleurage (extraction with cold fat): This method is carried out on a commercial scale only in France, and on a smaller scale than in the past. It is restricted to plants that, after harvesting, continue their physiological activities, forming and emitting flavors. Enfleurage gives a higher yield of oil than other methods. It has been replaced by extraction with solvents because it is a very delicate and time-consuming process, requiring experience and labor.
- Maceration (extraction with hot fat): This is applied to raw materials that give a low yield of oil when distillation or enfleurage are used. It has been almost entirely substituted by the organic solvent extraction process.
- Solvent extraction: This is applied to many different raw materials and is carried out in many countries. It is technically the most modern process in comparison to hydrodistillation, enfleurage, and maceration, yielding alcohol-soluble compounds.
- Supercritical solvent extraction: This technology can be applied to different raw materials and has the advantages of the absence of solvent residues in the extract and the use of lower temperatures. Moreover, it is less time-consuming when compared with the other techniques. At present, its main disadvantage is its high production cost, because of the pressures used.

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7

Distillation and Drying

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7.1 Distillation 157
7.1.1 General Aspects 157
7.1.2 Hydrodistillation 160
7.2 Drying 162
7.2.1 Drying Effects 166
References 167

7.1 DISTILLATION

7.1.1 General Aspects

Distillation is the most common and best known separation technique. It is widely used in chemical processing industries for the separation of components with different volatilities. It involves the creation of two or more coexisting phases, generally two – vapor and liquid – which differ in composition at the same temperature and pressure. These characteristics

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are used for the separation of the substances. Its costs may comprise more than 50% of a plant's operating costs.

According to Seader and Henley (1998), the art of distillation dates back at least to the first century A.D., when it was used to produce alcoholic beverages. As discussed by Heldman and Hartel (1998), distillation is used in the food industry for the separation of volatile components, including flavors from different matrices. For example, in the processing of orange juice, vapors coming off the evaporator contain a significant amount of volatile components that are separated from the condensed vapors in a distillation column.

Optimization of the distillation process is important. The control of distillation columns to reduce operating costs, to decrease energy consumption, and to improve the efficiency of separation is necessary. Up to the eleventh century, distillation consisted of a process in which the liquid to be separated was placed in a vessel to which heat was applied, causing part of the liquid to evaporate. The vapors from the heated vessel were condensed in another chamber, producing the distillate (Seader and Henley 1998). Today, distillation can be defined as an operation in which a mixture of substances, liquid and/or vapor, called feed, is separated into components of desired quality using heat as the separation agent. The separation is based on differences in the boiling point of the substances. The vapor phase becomes richer in components that have lower boiling points, and the liquid phase will become richer in the components that have higher boiling points. Many texts on the principles, operation, and mathematical modeling of distillation can be found in the literature (Coulson and Richardson 1991; McCabe and others 1993). For a more detailed approach, the book by King (1980) may be consulted.

Distillation columns and/or distillation operations can be classified according to many aspects. Focusing on the operation mode, batch or continuous operation can be performed. In batch operations, the column is charged with a determined amount of feed and, when the desired task is achieved, the column is opened, cleaned, and again charged with feed to start a new operational cycle. Each operational cycle is called a batch. In continuous operation (continuous columns), the feed stream flows continuously into the column, and the products are continuously withdrawn. In the continuous operation mode, distillation columns can process a large amount of materials, but in batch operation mode, only smaller amounts of materials are permitted. As a function of the number of components in the feed, there exist binary columns, where the feed contains two components, and multicomponent columns, where the feed contains more than two components. Multiproduct columns are those with more than two product streams. Based on the type of internal components in a column that promote contact between phases, the columns can be divided into two major types: tray (or plate) columns and packed columns. In tray columns, the liquid–vapor contact is promoted by bubbling the vapor phase through a liquid layer above each tray inside the column. There are several tray designs to better promote the liquid hold up and the vapor–liquid contact. In packed columns, the vapor–liquid contact occurs all along the column, and this contact is usually enhanced by filling the column with specially designed small objects that are dumped randomly into the column or with structured packings having an ordered geometry. It is known that, in principle, more theoretical trays might be obtained by removing the trays and replacing them with packing. In fact, more and more frequently, additional distillation capacity is being achieved with existing tray towers by replacing all or some of the trays with sections of packing. Packed columns have a higher surface area for liquid–vapor equilibrium to occur and provide more efficient separations, but with a lower throughput rate.

The separation of components with nearly the same boiling points is difficult by simple distillation. In such systems the separation can be improved by adding a third component.

In extractive distillation, the new component, with a higher boiling point and greater interaction with one of the original components in the liquid phase, enhances the separation by increasing the amount of this component in the bottom stream. In azeotropic distillation, the third component forms an azeotrope with one of the original components, resulting in a higher concentration of this component in the top stream. The stream containing the third component is an independent column feed. In extractive distillation, this extra feed is at the bottom of the column, and in azeotropic distillation, at the top of the column.

Distillation columns are made of several devices, each of which is used either to transfer heat energy or enhance material transfer. A typical distillation column contains several major devices: a vertical shell where the separation of liquid components is performed, internal column components such as trays/plates and/or packings, which are used to enhance component separations, a reboiler to provide the necessary vaporization for the distillation process, a condenser to cool and condense the vapor from the top of the column, and a reflux drum to hold the condensed vapor from the top of the column so that liquid (reflux) can be recycled back to the column. The vertical shell houses the internal column components and, together with the condenser and reboiler, constitutes a distillation column (also denominated a fractionator or still) (Tham 1997). A schematic representation of a conventional distillation column can be seen in Figure 7.1.

The feed material that is to be separated into fractions is usually introduced somewhere near the middle of the column to a tray known as the feed tray or at other points along the column shell. The feed tray divides the column into a top (enrichment or rectification) section and a bottom (stripping) section. The feed flows down the column, where it is collected at the bottom in the reboiler. Heat is supplied to the reboiler to generate vapor. The source of heat input can be any suitable fluid, although, in most chemical plants, it is usually steam. The vapor produced in the reboiler is reintroduced into the unit at the bottom of the column. The liquid removed from the reboiler is known as the bottoms product or residue, or simply bottoms. The vapor moves up the column, and, as it exits the top of the unit, it is cooled by a condenser. The condensed liquid is stored in a holding vessel known as the reflux drum. Some of this liquid is recycled back to the top of the column and is called the reflux. The condensed liquid that is removed from the system is known as the distillate or top product. Thus, there are internal flows of vapor and liquid within the column, as well as separation of components from a feed

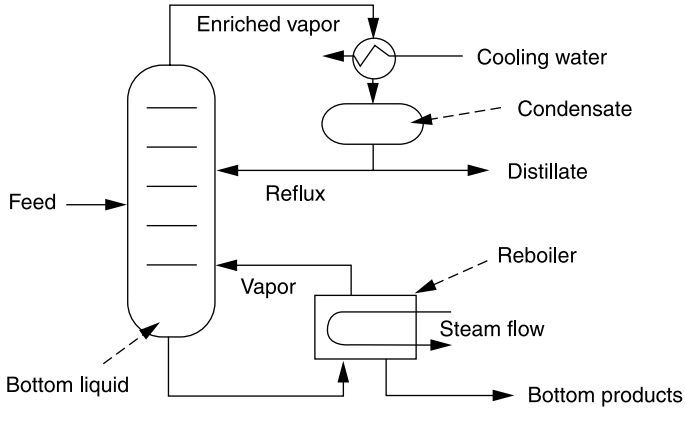

Figure 7.1 *Schematic of a conventional distillation column.*

mixture via distillation. The separation depends on the differences in boiling points of the individual components. In addition, the boiling point of the feed mixture will depend on the relative concentrations of the components. The distillation process depends on the vapor pressure characteristics of the liquid mixtures.

The design of distillation columns is based on the boiling-point properties of the components in the mixtures being separated. Thus the dimensions, particularly the height, of distillation columns are determined by the vapor–liquid equilibrium (VLE) data for the external flows of feeds and product streams into and out of the column, respectively.

Distillation involves the transfer of material from one phase to another. Often the residence time and intimacy of contact of the two phases in a separation device are not sufficient for the two phases to come to thermodynamic equilibrium with each other. The rate of mass transfer across the phase interface will govern the extent to which the phases equilibrate. Mass transfer rates between phases reflect the phenomenon of diffusion coupled with convective flow, turbulence, and gross mixing. The transferring component(s) must travel from the original phase to the interface and then from the interface to the new phase. For any real separation device, it will be necessary to correct an analysis based upon product equilibrium or ideal separation factors for the effects of entrainment, mixing, charging sequence, flow configuration, and mass and heat transfer limitations (King 1980). The most common way of correcting for these effects is to use the actual separation factor (which can be called column efficiency) that relates actual product compositions with the compositions based on the phase equilibrium.

7.1.2 Hydrodistillation

Distillation using water vapor is called steam distillation. Steam distillation can be used when the material to be distilled has a high boiling point and/or decomposition might occur if direct distillation were employed. The liquid is added to the still, and steam is passed through it. The solubility of steam in the liquid must be very low. Superheated steam that provides sufficient heat to vaporize the material concerned without condensing may be used. Otherwise, some of the steam may condense, producing a liquid water phase, as mentioned by Coulson and Richardson (1991).

Most essential oils have been obtained by steam distillation, or, in the more general sense, by hydrodistillation (first used by von Rechenberg as referring to codistillation with water vapor). The practical problems connected with distillation of aromatic plants are, therefore, of utmost importance to the actual producer of essential oils.

Essential, volatile, or ethereal oils are mixtures composed of a variety of volatile, liquid, or solid compounds that vary widely in concentration and boiling points. They are present in the interstices of vegetable tissues and can be extracted by hydrodistillation or by pressing. If extraction by pressing is used, the liquid mixture obtained usually has to pass through a separation process in order to remove undesired substances. At present, the most common process employed in this case is also hydrodistillation. Normally, these substances have a very low vapor pressure because they are high boiling substances. The intensity of the odor can be considered a manifestation of the volatility (related to the boiling point and vapor pressure) of the substance. Of course, there are many exceptions.

Little investigation has been undertaken on the process by which steam actually isolates the flavors present in the essential oils. It is commonly assumed that the steam penetrates the plant tissue and vaporizes all the volatile substances. If this assumption were

true, the isolation of oil from plants by hydrodistillation would appear to be a rather simple process, merely requiring a sufficient quantity of steam. However, this model is not always able to describe the extraction step in the process, and the complete prediction of the hydrodistillation process from a solid matrix by mathematical models is not possible even in a context of many theoretical models available for phase equilibrium of the substances involved.

Considering the manner in which the contact between water and the original matrix is promoted, a terminology that distinguishes three types of hydrodistillation has been developed: water distillation, steam distillation, and direct steam distillation. When the first method is employed, the material to be distilled comes in direct contact with boiling water. In the second method, the material is supported on a perforated grid or screen held some distance above the bottom of the still. In this case, low-pressure, saturated, wet steam rises through the material. The typical features of this method are that the steam is always fully saturated, wet, and never superheated, and the material is only in contact with steam, and not with boiling water. The last type of hydrodistillation, direct steam distillation, resembles the preceding type, except no water is kept in the bottom of the still, but live steam, saturated or superheated, is passed through the sample, and the process is frequently maintained at higher-than-atmospheric pressures.

Hydrolysis of certain components of the essential oils and decomposition caused by heat always occur in the hydrodistillation process. Although it is an inevitable reaction, the intensity of hydrolysis is typically low under the conditions normally used. The process temperature is determined entirely by the operating pressure, and its value is around 100°C at atmospheric pressure. At this temperature, decomposition by degradation of some substances occurs, and the decomposition products can cause interference in the odor of the oil. Considering these aspects, the temperatures used to extract essential oils by hydrodistillation should be kept as low as possible, and the contact between water and the original matrix should be minimized.

Hydrodistillation at high and reduced pressures and with superheated steam may be used for certain plant materials. Orris root, sandalwood, cloves, caraway seed, and pine needles, for example, are occasionally distilled with steam at a pressure that is higher than atmospheric pressure to obtain a more favorable ratio of oil to water in the distillate. The use of high-pressure steam for the rectification of volatile oils *per se* is not advisable, nor is it necessary, because superheated steam gives better results. Distillation of plant material with high-pressure steam should not be made a general practice, as it will increase the quantity of decomposition products in the plant material and in the oil. The degree of decomposition is influenced by temperature and the length of the distillation process. That is, steam distillation of plant material at high pressure is not advisable, because the higher temperature of the steam gives rise to decomposition products that impart an unpleasant odor to the oil. Nor is there any appreciable gain in the ratio of oil to water in the distillate, except perhaps in cases where the previous distillation under atmospheric pressure has been carried out inefficiently.

Steam distillation of plant material at reduced pressure may be subdivided into two types: steam distillation at slightly reduced pressure, which often shortens the length of the distillation process, and vacuum steam distillation at such a low pressure that the temperature remains just sufficiently above that of the cooling water so as to allow adequate condensation of the steam/oil vapors. The principal advantage of vacuum steam distillation consists in the purity of the flavor of the volatile oil obtained. It will be free from any off-flavor caused by decomposition, which accompanies most oils distilled above 70°C.

Because distillation is a very important separation process, much effort has been expended to increase the performance of existing distillation equipment and to develop new devices for facilitating vapor–liquid contact (Henley and Seader 1981). This effort can also be applied to more recently developed separation processes such as supercritical fluid extraction.

There are advantages and disadvantages for the methods for processing natural essential oils to concentrate aromatic components and for steam distillation of volatile oils. Some oils cannot be processed by hydrodistillation, because boiling water and steam have a deteriorating influence upon the rather delicate flavoring constituents. Certain raw materials yield no oil at all when distilled, and, hence, must be processed by methods other than distillation.

7.2 DRYING

Drying a solid generally refers to the removal of relatively small amounts of water or other liquid to reduce its final content from an initial value to some acceptable low value. The reduction in water or liquid content from solids can be achieved by mechanical (centrifugation or pressing) or thermal methods (vaporization). Often the mechanical removal of liquids from solids is performed before the thermal process, as it is less expensive and easier to use than thermal methods (Perry and others 1997; Sonaglio and others 1999). Mechanical methods for separating a liquid from a solid are not strictly considered drying, although they often precede a drying operation. This chapter is restricted to drying by thermal vaporization. In this case, the feed material is moist solids, the separating agent is heat, and the products are dried solid and water vapor. The solids to be dried may be in different forms, such as flakes, granules, crystals, powders, slabs, or sheets. The liquid to be dried may be on the surface of the solid, entirely inside the solid, or partly internal and partly external (McCabe and others 1993). Consequently, there are many types of dryers, differing basically in the way the solid is moved through the drying zone and in the way the heat is transferred. The choice of dryer is based on many factors, including characteristics of the material being dried (liquid, solid, particulate), economy of a particular dryer, capital cost of the dryer, and so on (Heldman and Hartel 1998). The drying operation always reduces the weight and volume and facilitates the milling of the materials (Mentz and Bordignon 1999).

In many cases, the drying of materials is the final operation in a manufacturing process, carried out immediately prior to packing or dispatching, and it often follows evaporation, filtration, or crystallization. In some cases, drying is an essential part of the manufacturing process, but, in the majority of the processing industries, according to Coulson and Richardson (1991), drying is performed for one or more reasons: (1) to reduce the cost of transport; (2) to make a material more suitable for handling; (3) to provide definite properties, such as maintaining the free flowing nature of salt; and (4) to remove moisture that may otherwise lead to corrosion. With some products, some care has to be taken. For example, it is essential that the crystals not be damaged during drying of a crystalline product; contamination of a pharmaceutical product must be avoided; and the loss of flavor from a fruit must also be prevented. Almost all the drying processes involve the removal of water by vaporization, requiring the addition of heat, and the major consideration is the effective utilization of that heat.

The moisture content of a material is usually expressed as a percentage of the mass of the dried material. If the material is exposed to air at a given temperature and humidity, the material will either lose or gain water until an equilibrium condition is established. This equilibrium moisture content varies widely with the moisture content and the temperature of the air. The moisture may be present in two forms:

1. *Bound Moisture* – this is water retained in such a way that it exerts a vapor pressure less than that of free water at the same temperature. Such water may be retained in small capillaries, adsorbed on surfaces, or as a solution in cell walls.
2. *Free Moisture* – this is water that is in excess of the equilibrium moisture content (Coulson and Richardson 1991).

The ability of air or hot gases to absorb the water liberated by evaporation will be determined by their temperatures and relative humidities.

The drying of natural raw materials is a complex phenomenon involving momentum, heat and mass transfer, the physical properties of the materials, air and water vapor mixtures, and the macro- and microstructure of the materials (Rizvi and Mittal 1992). There are many possible drying mechanisms, but those that control the drying of a particular product depend on its structure and the drying parameters, such as drying conditions, moisture content, dimensions, surface transfer rates, and equilibrium moisture content. These mechanisms fall into three classes: evaporation from a surface, flow as a liquid in capillaries, and diffusion as a liquid or a vapor (Rao and Rizvi 1995).

The mechanisms of internal flow are determined by the structure of the solid. The flow can be caused by gravity, by a sequence of vaporization–condensation, by shrinkage and pressure gradients, by capillary flow in granular and porous solids, and by diffusion in continuous, homogeneous solids. During drying, one mechanism predominates at any given time in a solid, but different mechanisms can predominate at different times during the drying cycle. According to Rao and Rizvi (1995), in the case of capillary-porous materials, water transport can take place by liquid diffusion caused by concentration gradients, liquid transport due to capillary forces, vapor diffusion due to partial vapor pressure gradients and liquid or vapor transport resulting from the difference in total pressure caused by external pressure and temperature, evaporation and condensation effects, surface diffusion, and liquid transport due to gravity.

In the typical dryer, moist solids are placed inside and heated by circulating air, heated walls, radiation, or microwave radiation. Heat must pass from the heat source to the particle surface and through the particle to wherever evaporation of water occurs. The water vapor generated must then travel to the surface of the particle and from the particle surface to a moisture sink, which may be a condenser, a desiccant, an exhaust of humid air, and so on (King 1980).

The application of the basic principles to the project for the dryer equipment is not direct. Variations in the drying conditions throughout the dryer, in the difference between the thermal and the mass transfer areas, in the configuration of the gas flow, in the effect of the operational conditions, and in the choice of the equipment according to the desired conditions of the dried products are among the difficulties encountered in the prediction of the drying velocity profiles. The selection of the dryer is usually based on preliminary tests, where the material is dried under the same conditions to be used in production.

The classification of dryers is not a simple task because of the very wide range of dryer designs available. Two steps are of primary importance for selecting a dryer for a

particular application: (1) Listing of the dryers that are capable of handling the material to be dried and (2) eliminating the more costly alternatives on the basis of annual costs (capital and operating costs). According to Perry and others (1997), dryers can be classified as direct or indirect. Direct dryers have the following characteristics:

1. Direct contact of hot gases with the solids is used for heating of solids and vapor removal.
2. Drying temperatures range up to 1000 K, the limiting temperature for most common structural metals. At the higher temperatures, radiation becomes an important heat-transfer mechanism, but it can cause degradation of the flavor compounds.
3. At gas temperatures below the boiling point, the vapor content of the gas influences the rate of drying and the final moisture content of the solid; with gas temperatures above the boiling point throughout, the vapor content of the gas has only a slight retarding effect on the drying rate and final moisture content (thus, superheated vapors of the liquid being removed can be used for drying).
4. For low-temperature drying, dehumidification of the drying air may be required when atmospheric humidities are excessively high.
5. A direct dryer consumes more fuel per pound of water evaporated and the lower the final moisture content, the greater the amount of fuel consumed.
6. The efficiency increases with an increase in the inlet-gas temperature for a constant exhaust temperature.
7. Because large amounts of gas are required to supply all the heat for drying, dust-recovery equipment may be very large and expensive when drying very small particles.

The indirect dryers differ from direct dryers with respect to heat transfer and vapor removal.

1. Heat is transferred to the wet material by conduction from an external medium such as condensing steam, usually through a metal surface with which the solid is in contact.
2. Surface temperatures may range from below freezing in the case of freeze dryers to above 800 K in the case of indirect dryers heated by combustion products.
3. Indirect dryers are suited to drying under reduced pressures and inert atmospheres to permit the recovery of solvents and to prevent the formation of explosive mixtures or the oxidation of easily decomposed materials.
4. Indirect dryers using condensing fluids as the heating medium are generally economical from the standpoint of heat consumption, as they furnish heat only in accordance with the demand made by the material being dried.
5. Dust recovery and dusty materials can be handled more satisfactorily in indirect dryers than in direct dryers.

As cited by Heldman and Hartel (1998), direct-contact dryers may be classified as fixed-bed or moving-bed. In fixed-bed dryers, the product is placed in the dryer, hot air is blown across the product to cause drying, and the product is removed when a certain moisture content is reached. Sun dryers, bin dryers, kiln dryers, or tray (cabinet or tunnel) dryers, which are typically batch dryers, are examples of this type of dryer. Moving-bed dryers, such as belt or conveyor dryers, belt-trough dryers, fluidized-bed dryers (Fig. 7.2), or rotary air dryers are continuous process dryers in which wet feed is

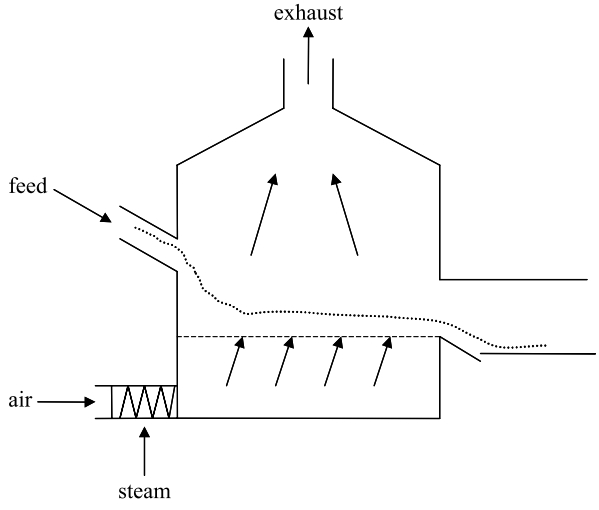

Figure 7.2 *Fluidized-bed dryer.*

input to the dryer and the final dried product is removed at the outlet. In the case of indirect-contact dryers, where heat is indirectly transferred from an external medium to the product through a heat-transfer surface, there is one common example, the drum dryer. The infrared contact dryer, where heat transfer occurs through a secondary mechanism, is another type of dryer. In each category, dryers can be operated either at ambient pressures or under vacuum to enhance vaporization at reduced temperatures. A final category of drying technology is freeze drying, where moisture is removed by a solid–vapor transition (sublimation) enhanced by low pressure.

There is no single theory of drying that covers all materials and dryer types. Many types of commercial equipment are available, with differences depending on variations in shape and size of stock, moisture equilibrium, mechanism of moisture flow through the solid, and method of providing the heat required for vaporization (McCabe and others 1993). The preliminary selection of dryers can be achieved by considering the following factors:

1. Properties of the material being handled (physical characteristics when wet and dry, corrosiveness, toxicity, particle size, abrasiveness);
2. Drying characteristics of the material (type of moisture, initial moisture content, maximum final moisture content, permissible drying temperature, probable drying time for different dryers);
3. Flow of material to and from the dryer (quantity to be handled per hour, continuous or batch operation, processing prior to drying, processing subsequent to drying);
4. Product qualities (shrinkage, contamination, uniformity of final moisture content, decomposition of product, over-drying, state of subdivision, product temperature, bulk density);
5. Recovery problems (dust recovery, solvent recovery);
6. Facilities available at the proposed site of installation (space, temperature, humidity, cleanliness of air, available fuels, available electric power, permissible noise, vibration, dust, heat losses, source of wet feed, exhaust-gas outlets).

7.2.1 Drying Effects

At the moment of harvest, an enzymatic degradation process begins in the plant that leads to degradation of the active principles. The incidence sunlight on the plants also accelerates the degradation of the substances present. A drying process that uses solar radiation promotes the degradation of the active principles, rapid drying of the edge of the vegetable tissue, and creation of an impermeable crust in these regions. In a relatively short time, the external surface of the material has a dried appearance, but its interior has a wet appearance.

The use of temperatures above room temperature is of extreme importance. For the drying of flowers and leaves, the temperature must be around 38°C. For barks and roots, temperatures up to 60°C are acceptable. The contact time varies around seven days at these temperatures (Mentz and Bordignon 1999). Temperatures over these limits accelerate the drying process, but promote the degradation of many volatile components (active principles). The drying of the plants must be achieved individually to avoid mixing the volatile components from each plant (Reis and others 1999).

The drying process will be faster if the material is present in small particles because of the greater surface area available for evaporation. For this same reason, the vegetable material must be placed in thin layers to optimize the drying process by facilitating transfer of moisture from the solid material to the environment (Mentz and Bordignon 1999). This procedure will allow greater efficiency and control of the operational conditions.

Hot-air greenhouses equipped with a thermostat may be used for drying, as the maintenance of a constant temperature during the desired period is guaranteed. It is also desirable to allow the air from the greenhouse to escape to prevent its saturation with the water vapor released from the drying material. The speed with which air circulates in the greenhouse has a great importance in the drying procedure. The use of greenhouse models provided with a system of forced air circulation is more efficient. The forced circulation provokes the constant renewal of air, removing the moisture-saturated air and facilitating the drying process.

Most liquid food flavorings are volatile and chemically unstable in the presence of air, light, moisture, and high temperatures. This is the case for most essential oils (Beristain and others 2001). The effect of drying on the compositions of the essential oils has been extensively studied. Mendes and others (2003) investigated the effect of natural and artificial drying on the composition of the essential oil of citronella and concluded that, in particular for this plant, the drying operation did not negatively influence the composition of the volatile compounds. In the case of the essential oil of lemongrass, Mendes and others (2004) observed large differences in the compositions of the essential oils obtained from dry and fresh leaves.

The perception of flavor in a food depends on the composition of its matrix. It has been shown that macromolecules, such as proteins, are involved in the retention of flavor compounds. The drying time may affect the retention of aroma; for example, the results obtained by Andriot and others (2004) have shown that the percentage of retention decreases significantly with the increase in freeze-drying time. Other studies are found in the literature with the same objective due to the fact that some plants and seeds are dried before they are processed (Radünz and others 2002).

Radünz and others (2003) studied the effect of temperature on the air drying of the extracted essential oil from guaco (*Mikania glomerata* Spengel) at 40, 55, and 70°C. It was observed that a temperature of 55°C did not influence the quantity of essential oil extracted and enabled a higher efficiency of extraction from the fresh plant in comparison to temperatures of 40 and 70°C.

The temperatures of 30, 40, 50, 60, and 70°C were investigated in the drying of citronella (*Cymbopogon winterianus*) plants (Rocha and others 2000). The results indicated that air drying at 60°C was best because of the shorter drying time and the higher efficiency of the essential oil extraction. This temperature did not affect the chromatographic profile or the quality of the essential oil.

Balladin and Headley (1999) studied the effects of drying methods using a wire basket solar dryer (temperatures not exceeding 50°C for 2 days) and an oven dryer (50°C for 2 h) on the composition of the essential oil of thyme (*Thymus vulgaris* Linné) herbs. The percentage of the essential oil was 0.5% using oven drying and 0.6% for the wire basket solar dryer method. The density of the extracted essential oil was lower than that of water. The difference was a result of the continuous heating in the oven for 2 h. In this case, some of the essential oils were lost. The authors suggested that the maximized use of supercritical extraction (using liquefied carbon dioxide) can yield higher concentrations of the volatile essential oils without thermal degradation.

A spray-dryer can be used to produce emulsions of essential oils using a gum as an encapsulating agent. Beristain and others (2001) studied the encapsulation of cardamom (*Elettaria cardamomum*) essential oil using this method. The conclusion of the work was that cardamom-based oil microcapsules were successfully produced by spray-drying using a gum. High flavor retention (83.6%) was attained during micro-encapsulation when a ratio of gum to oil equal to 4 was used.

Bos and others (2002) compared the volatile components present in both fresh and dried leaf and root material of *Anthriscus sylvestris* (L.) Hoffm. The essential oils were obtained through hydrodistillation and by extraction with dichloromethane, and the plants were submitted to air- and freeze-drying (lyophilization). Lyophilization resulted in more loss of volatile constituents than drying at room temperature. There were also quantitative differences between these extracts. In addition, air-drying and freeze-drying resulted in a significant loss of volatile constituents as compared to the fresh material.

Jerkovic and Mastelic (2003) have isolated volatile components from fresh and air-dried leaf buds of *Populus nigra* L. (Salicaceae). Forty-eight components were identified. Fresh buds contained 0.27% essential oil, and dried buds contained 0.12%. The authors concluded that the reduction in the amounts of volatile substances during the drying process depended on the volatility (physical–chemical properties) and the botanical structure of the particles that store the essential oil. Although the leaf buds contained numerous nonpolar compounds (waxes and lipids), a significant loss of volatile compounds was noted in dried leaf buds. Air-drying moderately effected the qualitative and quantitative composition of polar bud volatiles. It is well known that drying plant material could also have an effect on glycosides, as the enzymes are still active for some time after plant collection and could catalyze hydrolysis.

As drying is a unit operation that affects the composition of some essential oils, affecting the quality and characteristics, operational conditions such as temperature and velocity of the air-drying must be controlled. Thus, drying is necessary to reduce the moisture content, but more studies have to be made to better control the processes involving plants, seeds, and so on, without damaging the flavoring compounds.

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8

Genetic Engineering

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8.1 Introduction 169
8.2 Genetics and Molecular Genetics in Vegetables for Flavor Production 170
8.3 Genetic Engineering of Microorganisms and Flavor 171
8.4 Advances in Beer Flavor using Genetic Tools 174
8.5 Future Prospects 176
References 176

8.1 INTRODUCTION

The knowledge that bacteria and viruses have genes that could mutate gave rise, in the 1940s, to the discovery of the genetic information present in DNA molecules. Interdisciplinary applications of very different novel research strategies explain the rapid progress in life sciences in the middle of the twentieth century.

The rising popularity of natural products during recent decades has triggered significant research activities in the use of bioprocesses for the production of flavor compounds. Products derived from bioprocesses starting with natural substrates are defined as natural if they have already been identified in plants or other natural sources. Natural flavors extracted from plant materials are often either too scarce or too expensive for

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commercial use. Commercial exploitation of biotechnology in this area relies not only on technical advances, but also on satisfying certain regulatory considerations.

The flavor industries worldwide are expected to expand at an increased rate. Natural flavors and their ingredients are the largest growing sector of this market. Biotechnological production of flavor compounds can be carried out through a number of different processes, among them plant cell and tissue culture, biocatalysis and biotransformation with microorganisms, or *de novo* synthesis (Marasco and Dannert 2003).

This chapter focuses on the application of genetic engineering for the synthesis of natural flavor compounds. The intention is to present some examples of bioprocesses currently used or under investigation.

8.2 GENETICS AND MOLECULAR GENETICS IN VEGETABLES FOR FLAVOR PRODUCTION

Terpenoids comprise a diverse class of natural products from which several commercial flavors, fragrances, and medicines are derived. These compounds give our foods their wonderful aromas and tastes, and many of our cleaning reagents their fresh scents. These products are commonly isolated from plants, microorganisms, and marine organisms. Because these compounds are naturally produced in small quantities, purification from biological material results in low yields and consumption of large amounts of natural resources.

Terpenes and sterols are complex molecules synthesized by equally complex biosynthetic pathways. Plants are known to accumulate a diverse range of sterols and triterpenes, and there has been considerable effort to unravel terpene metabolism in plants. Biologically, the terpenes are very important compounds for plants. They provide unique means acting as hormones in plants, orchestrating development programs. Impressive progress in understanding these complex biochemistries has opened up opportunities to use genetic engineering to alter terpene metabolism for industrial applications.

The isoprenoid or terpene biosynthetic pathway in plants is probably one of the most complex and elaborate. It consists of two pathways operating independently of each other and localized in separate intracellular compartments. To simplify, the mevalonate pathway (MEP) provides a good route to manipulate the synthesis of these compounds in plants using the tools of genetic engineering.

Although there has been some success in manipulating the cytosolic mevalonate pathway, recent progress by Lewinsohn and others (2001) and Mahmoud and Croteau (2001) represents strides forward in manipulating the MEP (Fig. 8.1). The work of these groups addresses a broader conceptual issue of how the flux of carbon down the MEP is controlled or regulated.

The challenge in manipulating flavors and fragrances is not to divert too much of the carbon entering the MEP, but just enough to produce sufficient flavor molecules, allowing their detection by the olfactory senses.

Lewinsohn and others (2001) argued that even when only a small amount of geranyl pyrophosphate (GPP) is available in developing tomato fruits, a properly engineered monoterpene synthase might be able to siphon away enough carbon from the MEP pathway for the production of volatile monoterpenes. These investigators fused a linalool synthase gene from *Clarkia breweri* that had been well characterized previously to the E8 promoter element known to direct gene expression during fruit development. They could

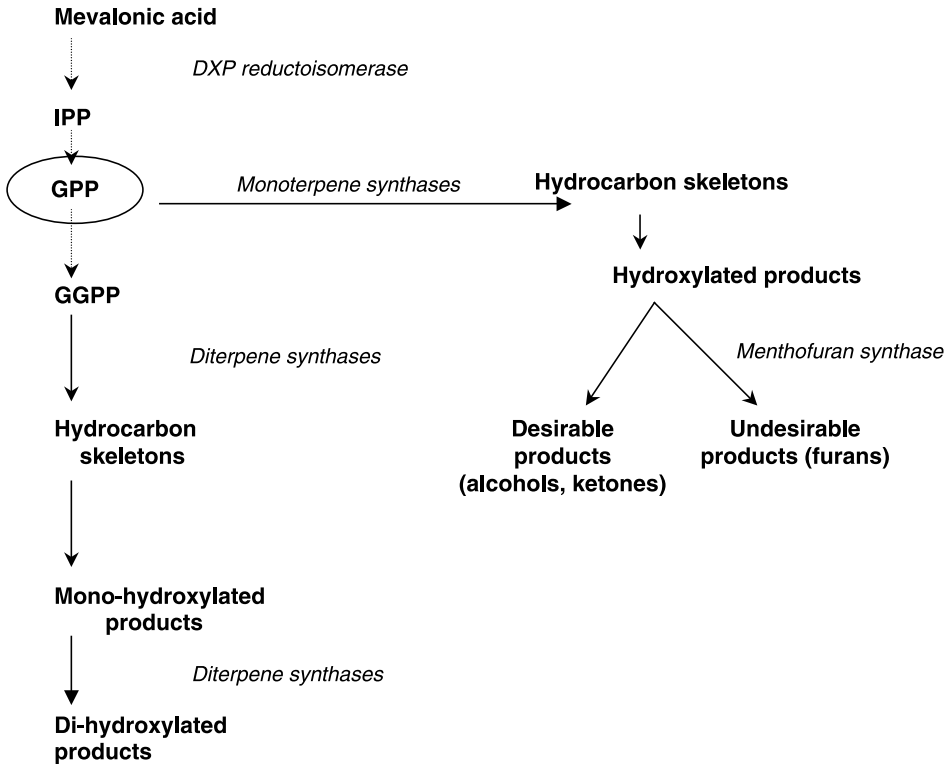

Figure 8.1 *The mevalonate pathway.*

observe that the transgenic fruits not only accumulated linalool during ripening, but also a hydroxylated derivative of linalool at 25–50% of the linalool present.

Mahmoud and Croteau (2001) introduced the gene encoding 1-deoxy-D-xylulose-5-phosphate (DXP) reductoisomerase into peppermint under the control of a strong constitutive promoter, and examined the complete terpene profile resulting from these transgenic lines. This gene limited an early step in the MEP that resulted in a 30% increase in the total content without changing the monoterpene profile. These investigators also demonstrated the feasibility of a new approach to enhance the quality of peppermint oil. Monoterpene hydrocarbons derived from the action of monoterpene synthases can be modified in different ways. In peppermint, menthofuran is derived from such modification of monoterpenes and is considered undesirable because it alters the flavor and color characteristics of the oil. Upon expressing an antisense form of the menthofuran synthase gene in transgenic peppermint, these authors observed a reduction of 50% in the menthofuran content, without any alteration in the terpene profile of the oil.

8.3 GENETIC ENGINEERING OF MICROORGANISMS AND FLAVOR

As for every bioprocess, the screening of suitable microorganisms, the adjustment of a full set of chemical and physical parameters, the design of the reactor, and a reliable online monitoring must be considered (Krings and Berger 1998).

Recombinant DNA technology has allowed low-cost production of flavor compounds. Genetic engineering of recombinant microorganisms is useful for improving the industrial production of flavor from microbial biotechnology. Functional expression of newly discovered flavor-generating plant enzymes in bacteria or yeasts constitutes a potential starting point for a bioprocess-oriented optimization.

Lactones are important flavor components of foods, responsible for the pleasant flavor of a variety of fruits and fermented foods. Lactones have been described as fruity, coconut-like, buttery, sweet, or nutty. A variety of microorganisms can perform *de novo* biosynthesis.

In the case of γ-decalactone (DECA), which has a peachy and creamy flavor, the basic process involves the biodegradation of ricinoleic acid using *Yarrowia lipolytica*, as indicated in Figure 8.2. However, other lactones have no sensorial properties or exhibit

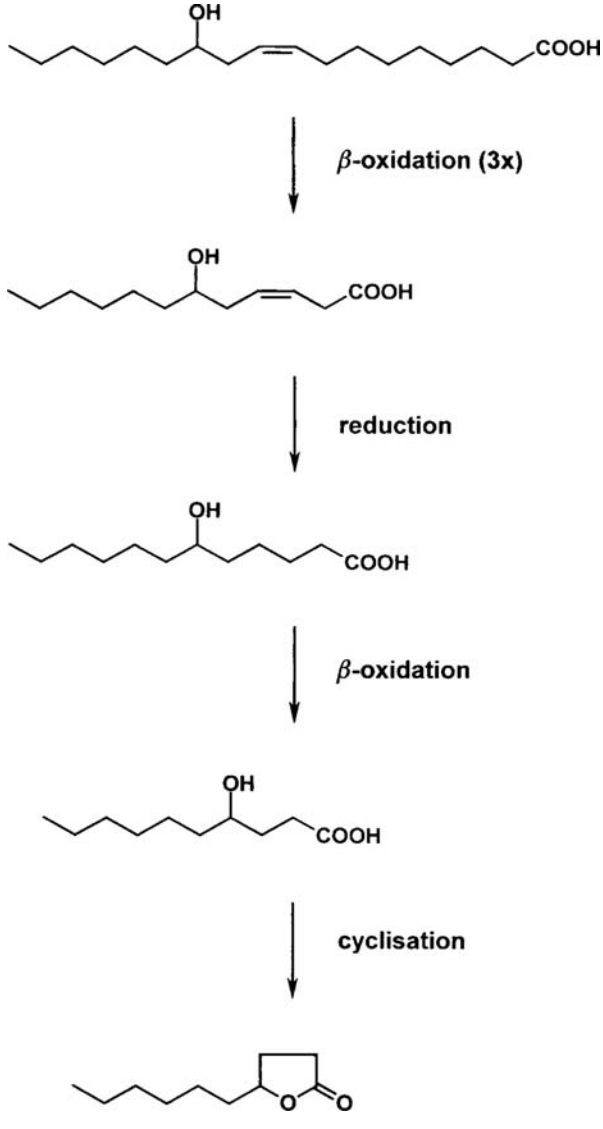

Figure 8.2 *The biodegradation of ricinoleic acid.*

mushroom or fruity notes. Furthermore, these lactones are produced at a higher rate and complicate the extraction of DECA.

The yeast *Y. lipolytica* possesses a five-gene family coding for acyl-CoA oxidase coding for acyl-CA oxidase (Aox1 to Aox5), the first enzyme of the pathway that is generally considered as catalyzing the rate-limiting step. It is possible to optimize the β-oxidation pathway of *Y. lipolytica* for production of DECA by modifying the genotype of the yeast to block β-oxidation at the C10 Aox level (Wache and others 2002). A strain not capable of degrading C10 was constructed so as to accumulate the precursor of DECA. Twice the amount of DECA was obtained from the mutant strain. Although this strain was efficient for lactone production, it was not growing as rapidly as the wild type on fatty acids. Using genetic engineering, it was possible to construct a yeast strain growing at a good rate and able to produce DECA by blocking the activity of Aox4. It produced 10 times more lactone in 48 h compared to the wild type, and this amount did not significantly decreased in 250 h (Groguenin and others 2004).

Citrus fruits possess unique flavors rarely found in other fruit species. Fruit flavor is a complex combination of soluble and volatile compounds. Several low-abundance sequiterpenes, such as valencene, nootkatone, and alpha- and beta-sinensal, are important flavor and aroma compounds in citrus (Sharon-Asa and others 2003).

D-Limonene (4-isopropenyl-1-methylcyclohexene) is one of the most widely distributed monoterpenes and is biosynthesized by more than 300 different plants (Menendez and others 2002). D-Limonene is the major component of peel oil in orange and lemon. Because of its low cost and extensive availability from citrus waste, this monoterpene is a very convenient starting material for bioconversions to higher value flavor and fragrance constituents.

Some microorganisms are able to convert limonene to chemicals of interest, including α-terpineol, carvone, and perillyl aldehyde and alcohol. *Bacillus stearothermophilus* can utilize D-limonene as the only carbon source for growth. Usually, aerobic thermophilic bacteria have rapid growth rates, broad metabolism, and resistance to chemical toxicity (Gurajeyalakshmi and Oriel 1989). The capability of *B. stearothermophilus* to grow on limonene may result in the production of monoterpenes of interest. The cultivation of *B. stearothermophilus* BR388 on minimal medium supplemented with 0.0125% yeast extract and limonene resulted in the formation of multiple metabolites such as perillyl alcohol, α-terpineol, perillyl aldehyde, and cyclohexanol (Chang and Oriel 1994).

To better understand the limonene pathway and the enzymes participating in monoterpene metabolism, the pathway encoding limonene metabolism was cloned as a 9.6 Kb chromosomal fragment from *B. stearothermophilus* BR388 into *Escherichia coli* (Chang and Oriel 1994). Limonene was the only carbon source. It resulted in the bioproduction of α-terpineol, perillyl alcohol, and perillyl aldeyde.

Studies on how to improve the bioconversion of limonene into special flavor compounds show the importance of limonene hydroxylase. This enzyme proved to be capable of limonene hydroxylation into a mixture of carveol and perillyl alcohol as well as dehydrogenation of these products to carvone and perillyl aldehyde.

A chromosomal DNA fragment of 3.6 Kb from *B. stearothermophillus* BR388 into *E. coli* has been sequenced, revealing a single open reading frame encoding a single subunit of limonene hydroxylase containing 444 amino acid residues. Using *E. coli* cells overexpressing limonene hydroxylase resulted in perillyl alcohol and carvone as principal products (Cheong and Oriel 2000).

Bioindustries are discovering the importance of biooxidation in fermentation processes. It allows the introduction of oxygen into nonactivated carbon atoms in a sterically and optically selective way.

Cytochrome P-450 monooxygenases can be isolated from bacteria, yeasts, insects, mammalian tissues, and plants. They play an essential role in the biosynthesis of numerous secondary metabolites and they can be efficiently expressed in *E. coli* cells. All microbial P450 monooxygenases that are expressed keep their activity during production and isolation (Urlacher and others 2004). The cytochrome P450 from *Pseudomonas putida*, which is a camphor-hydroxylating enzyme, was engineered to enhance activity towards α-pinene. The resulting products were (+)*cis*-verbenol and (+)verbenone, which are natural fragrances and flavors (Bell and others 2003).

Carotenoids form a large group of structurally diverse higher terpene pigments, widespread in plants and microorganisms. Potent flavor compounds are derived from carotenoids. These diverse compounds are the result of the oxidative cleavage reactions of carotenoid compounds into the corresponding aldehydes. Volatiles such as β-ionone, α-ionone, dihydroactinidiolide, damascenol, and β-cyclocitral are obtained from the cleavage of 9–13 carbon carotenoids (Glória and others 1993). The ionones are found in many fruits, such as raspberry, blackberry, peach, melon, and tomato.

Recently, Zorn and others (2003) performed a screening of potential β-carotene degrading strains. Four strains produced dihydroactinidiolide, and three others produced β-ionone as the main product, along with β-cyclocitral, dihydroactinidiolide, and 2-hydroxy-2,6,6-trimethylcyclohexanone.

The increasing amount of genome sequence data gives access to new enzymes including those playing key roles in plant flavor biosynthesis. The construction of microbial host vector systems simplifies the functional expression and molecular biochemical characterization of these enzymes. Isolation, cloning, and expression of plant genes into microbial hosts may be the key to natural flavor production.

8.4 ADVANCES IN BEER FLAVOR USING GENETIC TOOLS

Volatile substances such as alcohols, esters, sulfur compounds, and phenols are important for beer quality and in controlling the formation of the volatile flavor profile. To achieve effective control, the mechanism of production of these compounds should be understood. Two major areas are in development: the first is the physicochemical methodology used to determine and quantify flavor components during the fermentation process, and the second is yeast genetic engineering (Hammond 1995). As a result, more than 530 beer flavor compounds have been identified (Maarse and Visscher 1998).

Although raw material such as hop and malt are important factors for beer flavor development, there is a general agreement that flavor compounds are mostly produced by yeasts during fermentation. For the moment, none of the genetically engineered brewer's yeasts is in use because of problems in obtaining public acceptance and lack of governmental regulations. In the following paragraphs some examples of yeast genetic engineering modification to improve beer flavor are discussed.

Diacetyl is an off-flavor easily found in beer. The diacetyl pathway is shown in Figure 8.3. Diacetyl is formed from acetolactate, an intermediate in the synthesis of the amino acids isoleucine and valine (Inoue and others 1968; Inoue and Yamamoto 1970). Most of the acetolactate is converted into valine, but a part is converted into diacetyl by

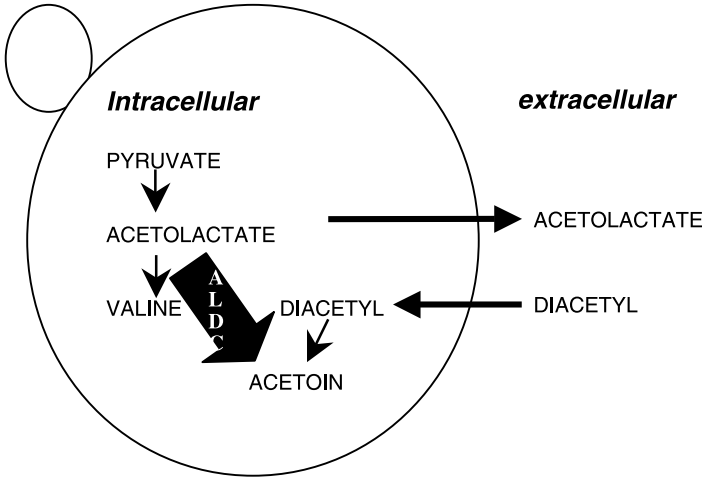

Figure 8.3 *The diacetyl pathway.*

nonenzymatic oxidation. Diacetyl is reduced by yeasts to acetoin by diacetyl reductase during maturation. Acetoin has no effect on beer flavor. There are several approaches to alter the concentration of acetolactate in the fermentation process (Hansen and Brandt 1996). The conversion of acetolactate to diacetyl and subsequently to acetoin can be limited by a shorter maturation period.

The enzyme acetolactate decarboxylase (ALDC) converts acetolactate directly to acetoin. ALDC is found in some microorganisms, but not in yeasts. This enzyme was introduced into brewer's yeast to accelerate beer maturation and to reduce the acetolactate concentration in young beer (Sone and others 1999). The ALDC gene was cloned from *Acetobacter aceti*, which is used in vinegar production (Yamano and others 1994). The gene was linked to the promoter and terminator of the *Saccharomyces cerevisae* phosphoglycerate kinase gene for expression. In that way, the ALDC expression cassette was integrated into the genome of commercial brewer's yeast. The fermentation performances of the parent yeast and the transformant were almost identical in terms of sugar consumption and flavor volatile production. However, diacetyl formation by the transformant was reduced to 10–20% of that produced by the parent. Other tests confirmed that the beer produced by the transformant had the same flavor and quality (Tada and others 1995). A similar result was obtained using the *Enterobacter* ALDC gene (Sone and others 1988; Suihko and others 1989). These results indicate that genetic engineering of brewer's yeast is an effective tool to improve beer flavor and quality.

Besides the reduction of the formation of a flavor compound, it is also possible to modify the metabolism to increase the formation of certain compounds. Higher alcohols and esters are formed by the yeast during wort degradation, and some of these compounds contribute significantly to the taste and flavor of beer. Isoamyl and isobutyl alcohol and their acetate esters are especially important flavor constituents in beer. These alcohols are formed from the corresponding keto-acids α-ketosivalerate and α-ketoisocaproate by addition of α-keto acid dehydrogenase. These keto acids may be formed either through branched-chain amino-acid biosynthesis or through deamination of valine and leucine. There are different approaches to increase the formation of the isoamyl esters

in beer. One of them is by controlling the esterase activity to increase the level of esters. Watanabe and others (1993) induced in mutants of sake yeast resistance to isoamyl monofluoroacetate, which resulted in an 85% reduction of esterase activity and 1.5–2.0 times increase in formation of isoamyl and isobutyl acetate.

8.5 FUTURE PROSPECTS

Innovative strategies for biocatalytic flavor generation will certainly take advantage of the emerging fields of functional genomics, proteomics, protein and metabolic engineering. Genetic engineering in flavor biosynthesis will become the most useful way to obtain natural flavors. As an increasing amount of genome sequence data becomes available, the improved genomic and proteomic methodologies provide access to new enzymes, including those playing key roles in plant flavor biosynthesis.

Operation under mild conditions to protect unstable products, that is, green processes, and functionalization of complex precursor molecules in a highly specific way (chemo-, regio-, and stereospecific) are important factors allowing biocatalysis to outplay chemical approaches in certain cases. Research exploiting these advantages of flavor biocatalysis will certainly be intensified in the future.

Finally, the industrial application of novel biocatalysis strategies will depend upon a less prejudiced and more balanced public perception of the use of genetic engineering for improved food quality and more environmentally friendly production processes (Schrader and others 2004).

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9

Flavor Compounds Produced by Fungi, Yeasts, and Bacteria

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9.1 Introduction 180
9.2 Main Technologies 180
9.3 How to Obtain Flavors 180
9.3.1 Flavor from Fermented Foods 180
9.3.1.1 Dairy Products 181
9.3.1.2 Alcoholic Beverages 181
9.3.1.3 Bakery Products 183
9.3.1.4 Mushroom Flavors 183
9.3.2 Biosynthesis of Flavor Compounds 183
9.3.2.1 Esters 183
9.3.2.2 Aldehydes 186
9.3.2.3 Alcohols 186
9.3.2.4 Carbonyls 186
9.3.2.5 Terpenes 187
9.3.2.6 Lactones 187
9.3.2.7 Pyrazines 187
9.3.3 Enzymatic Technology 188
References 189

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

9.1 INTRODUCTION

Microorganisms play an important role in the generation of natural compounds, particularly in the field of food flavors. For a long time, plants were the sole sources of flavor compounds and most of them were isolated from essential oils. However, active compounds are present in low concentrations, which makes their isolation difficult. Another disadvantage of plants as a source of flavors is the dependence on factors that are difficult to control such as the weather and the risk of plant diseases. The production of flavor compounds by biotechnological methods has been an interesting alternative due to consumers' preference for natural ingredients. Microbial processes seem to be the most promising methods for the production of natural flavors.

9.2 MAIN TECHNOLOGIES

Many microorganisms are capable of synthesizing flavor compounds when growing on a culture media. They have the ability to perform conversions that would require multiple chemicals steps. Microorganisms are used to catalyze specific steps. They are also an economical source of enzymes, which can be utilized to enhance or alter the flavors of many food products (Kempler 1983). In this way, biotechnological processes involved in the production of flavor compounds can be divided into two groups: microbiological and enzymatic. Microbiological methods are subdivided into biosynthesis and biotransformation. The first is the production of chemical compounds by cells (fermentation or secondary metabolism). The second refers to the use of microbial cells in the specific modification of chemical structures (Welsh and others 1989).

In fermentation, the production of flavors starts from cheap and simple sources such as sugars and amino acids. The product is generated by the complex metabolism of the microorganism. When microorganisms are used in order to catalyze specific conversions of precursors and intermediates, the process is called biotransformation. Although fermentation requires C and N sources, a specific substrate is necessary for microbial transformation. The enzymatic catalysis precedes a simple and specific transformation of the substrate molecule. The substrate does not have to be "natural"; according to Schreier (1989) "non-natural" substrates can also be biotransformed.

It is important to distinguish research with the purpose of obtaining complex products with natural characteristics from those that try to obtain isolated molecules. The first consists in the experience of nature imitation and in developing a process with one or more microorganisms and enzymes. The second tries to obtain a higher yield of the characteristic components. The choice between them determines the methodology, which will be employed *in vivo* or *in vitro*, through biosynthesis or bioconversions (Delest 1995).

9.3 HOW TO OBTAIN FLAVORS

9.3.1 Flavor from Fermented Foods

The sensory properties of fermented foods are one of the key parameters in distinguishing these products from foods that have undergone undesirable spoilage. The organoleptic properties of fermented foods usually differ from those of the unfermented substrate

and are dependant upon the biochemical activities of the associated microorganisms (Cook 1994).

Fermentation has been practiced for the production of food since ancient times. It has become an effective technology for the production of organic acids, flavor compounds, and other biologically important chemicals. New aroma and flavors includes acids, alcohols, carbonyl compounds, esters, and pyrazines.

The use of microorganisms in the production of food has been practiced for a long time to improve the sensory quality of the food. Products such as beer, wine, distilled beverages, bakery, vinegar, fermented vegetables, milk, soybean, and meat are preserved, modified, and flavored using microorganisms. The flavor compounds of traditionally fermented foods originate from a complex microflora that acts in the chemical precursors of a food matrix (Berger 1995).

Lactic acid and alcoholic fermentations are the two important processes responsible for fermented food flavors. However, in some cases, the flavor is formed by specific fermentations (Joshi and Pandey 1999). The creation of new fermented products can result in the development of novel flavors and textures.

9.3.1.1 Dairy Products. Cheese flavors find application in snacks, sauces, baked goods, and several other products. Yogurt and buttermilk flavors are also useful. The cheese flavor results from the action of microorganisms and enzymes on milk's proteins, fats, and carbohydrates. Numerous breakdown products are formed, among them, short-chain fatty acids, acetic and lactic acids, alcohols, aldehydes, ketones, esters, sulfur and nitrogen compounds (Sharpell 1985). Marilley and Casey (2004) have reported that the use of bacteria strains for cheese ripening with enhanced flavor production is promising. They also mentioned that the catabolism of amino acids is presumably the origin of some major flavor compounds.

The starter cultures used in dairy technology are mainly prokaryotes like *Lactococci*, *Lactobacilli*, *Leuconostocs*, *Bifidobacteria*, *Propionibacteria*, *Streptococci*, and *Brevibacterium linens*.

Yeasts, such as *Kluyveromyces*, *Debaromyces*, *Candida* or *Trichosporon* are present in many manufactured milk products. These microorganisms modify the sensory characteristics of the products by synthesizing or assimilating volatile nitrogen and sulfur compounds.

Several chemical reactions take place in the surface of ripened cheeses such as Camembert and Brie during maturation due to fungi growth. The Fungal mycelium of *Penicillium roqueforti* grows rapidly and the resulting products are used directly for flavoring foods with a blue cheese-type flavor.

9.3.1.2 Alcoholic Beverages. Flavor compounds are produced as byproducts of yeast metabolism during alcoholic fermentation. Many flavor compounds have been identified in alcoholic beverages. The main compounds are listed in Table 9.1.

During alcoholic fermentation, yeasts transform sugars (glucose, fructose, and sucrose) into ethanol and carbon dioxide by the Embden–Meyrhof–Parmas pathway. This is the main bioreaction, but not the only one and, at the same time, several secondary byproducts are formed. Higher alcohols, organic acids, and esters are the main flavor compounds.

Higher alcohols, which contain more than two carbons, are also called fusel alcohols. They constitute the major portion of the secondary products of yeast metabolism. They

TABLE 9.1 Compounds Produced by Yeasts During Alcoholic Fermentation.

Class	Compounds
Esters	Amylacetate, butyl acetate, ethyl acetate, ethyl butyrate, ethyl lactate, ethyl benzoate, ethyl hexanoate, ethyl guaiacol, ethyl-2-methyl butyrate, ethyl-3-methyl butyrate, ethyl octanoate, ethyl octenoate, ethyl decanoate, ethyl dodecanoate, diethyl succinate, 3-methyl propionate
Alcohols	Ethanol, 2-methyl butan-1-ol (amyl alcohol), methyl butan-1-ol (isoamyl alcohol), heptanol, hexan-1-ol, 2-phenyl ethanol, 2-methyl propanol, glycerol, 2,3-butanediol, n-propanol
Carbonyls	Decalactona, decan-2-one, acetaldehyde, butyraldehyde, hexanal, nonanal diacetyl benzaldehyde
Acids	Acetate, butyrate, lactate, malate, succinate, hexanoate, nonanoate, octanoate
Sulfur derivatives	Methionol, ethanetiol, methylthioacetate, dimethyl disulfide, ethyl methyl disulfide, diethyl disulfide, 3-methylthiopropyl acetate, 2-mercaptoethanol, *cis*- and *trans*-2-methylthiophanol
Phenolic compounds	Vinyl phenol, ethyl phenol, ethyl guaiacol, vinyl guaiacol

include n-propanol, isobutyl alcohol, 2-methyl butanol, amyl alcohol, isoamyl alcohol, and 2-phenyl ethanol. Isoamyl alcohol accounts for more than 50% of the total concentration of higher alcohols.

Esters at appropriate concentrations impart flowery and fruity flavors. They are formed by esterification of fatty acyl-CoA or of organic acid by alcohols. Esters are present in very low amounts, near their threshold level. However, ethyl acetate has been found in wine in high concentrations.

Beer. Acetaldehyde, the most important aldehyde in beer, is formed as a metabolic branch point in the pathway from carbohydrate to ethanol. Its level varies during fermentation and ageing of beers, reaching 2–20 mg/L. At concentrations of 20–25 mg/L, acetaldehyde causes "green" or "vegetable" flavor.

Diacetyl and pentane-2,3-dione (vicinal diketones) have a characteristic flavor described as "buttery", "honey" or "toffee-like". They have a very high off-flavor potential, dependent on the fermentation temperature. The threshold for diacetyl in lager-type beers is 0.10–0.14 mg/L. At levels above 1 mg/L, it becomes increasingly "cheese-like" and sharp.

Volatile acids are usually present in beer at concentrations of 20–150 mg/L. Butyric and iso-butyric acids in concentrations of 6 mg/L cause a "butyric" or "rancid" flavor. Valeric and iso-valeric acids cause an "old-hop" and "cheesy" flavor. Fatty acids with 6 to 12 carbon fatty acids give the characteristic flavor of "cheesy", "goaty", or "sweaty" (Smogrovicová and Dömény 1999).

Wine. The chemical composition of wine is determined by many factors, among them grape variety, geographical and viticultural conditions, microbial ecology of the grape, fermentation processes, and winemaking practices. Microorganisms affect the quality of the grape before harvest and during fermentation. They metabolize sugars and other components into ethanol, carbon dioxide, and hundreds of secondary products that contribute to the characteristic flavor of wine (Fleet 2003).

9.3.1.3 Bakery Products. Although *Candida* yeast has occasionally been used for baking and some *Saccharomyces carlsbergensis* strains have been patented for use as baker's yeast, pure strains of *Saccharomyces cerevisae* are almost universally employed.

9.3.1.4 Mushroom Flavors. The commercially important mushrooms belong to the orders *Ascomycetes* and *Basidiomycetes*. Truffles (*Tuber* sp.) and morels (*Morchella* sp.) represent the *Ascomycetes*. *Basidiomycetes* are represented by *Agaricus bisporus*, *A. bitorquis*, *Lentinus edodes* (Shiitake), *Volvariella volvacea*, *Pleurotus* sp., and *Flammulina velutipes*. The main chemical compound responsible for the mushroom flavor is 1-octen-3-ol, although several others, including glutamic acid and 5′-guanylic acid, can modify the flavor, giving each mushroom species its distinctive characteristic. There is interest in growing mushroom mycelium in submerged culture and then utilizing the dried mycelium as a flavor compound (Sharpell 1985).

9.3.2 Biosynthesis of Flavor Compounds

Biochemical reactions as well as several nonenzymatic reactions involving sugars, fatty acids, and amino acids give rise to flavor during fermentation. Several reports and reviews have been published on the production of volatile compounds by microorganisms (Janssens and others 1988, 1992; Berger 1995; Jiang 1995; Christen and others 1997; Bramorski and others 1998; Soares and others 2000; Medeiros and others 2001). Although several bacteria, yeasts, and fungi have been reported to produce flavor compounds, a few species of yeasts and fungi are often preferred. However, only a few of them find application in the food industries due to their GRAS (Generally Recognized As Safe) status.

Flavor compounds derived from microorganisms are often produced in low concentrations. These compounds have low thresholds and can be detected by chromatographic methods in parts per million (ppm, μL/L). The amount and type of compounds secreted by microorganisms depend on the strain, with its enzyme-specific action, chemical composition of the culture medium, pH and temperature control, age of inoculum, and water activity of the substrate.

Flavor compounds produced by *Trichoderma viride, Penicillium roqueforti*, and *Penicillium decumbens* have been detected during the phases of growth or sporulation, depending on the culture medium (Latrasse and others 1985).

Singhal and Kulkarni (1999) presented a schematic representation of the flavor compounds produced by microorganisms (Fig. 9.1). Some examples of flavor compounds produced by microorganisms are also indicated in Table 9.2.

9.3.2.1 Esters. Esters are a very important class of flavor compounds of fresh fruits and fermented foods, which are found in concentrations between 1 and 100 ppm (Janssens and others 1992). The production of the ester ethyl acetate by the yeast *Candida utilis* from glucose is observed when the yeast grows on a medium containing a specific initial ethanol concentration.

Esters of low molecular weight are responsible for fruity odors and consist of acids and their derived compounds such as acetates, propionates, and butyrates. Some examples are ethyl butyrate and isoamyl acetate, which are found in strawberry and banana (Macedo and Pastore 1997).

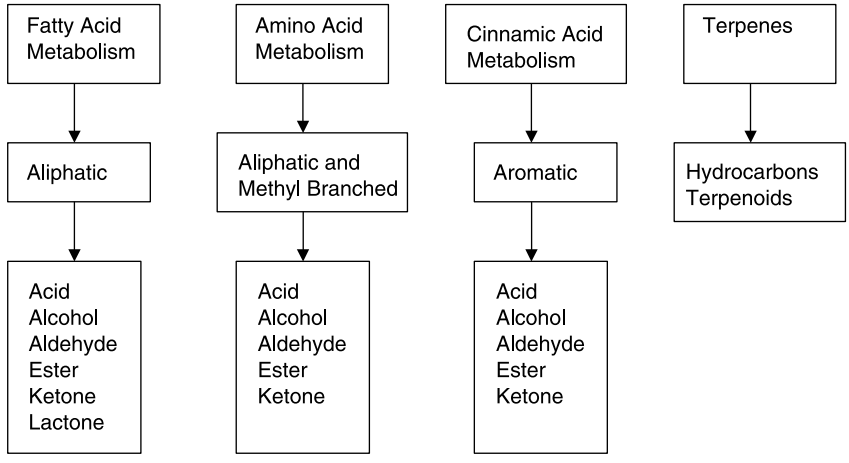

Figure 9.1 *Flavor compounds produced by microorganisms.*

The presence of esters such as ethyl acetate and butyric acetate in the culture medium can eventually describe a detoxification mechanism by which the microorganism avoids the accumulation of toxic compounds. The production of acetates occurs in order to detoxify the medium by converting acetic acid and high alcohols (Latrasse and others 1985).

TABLE 9.2 Microbial Production of Flavor Compounds.

Compounds	Microorganism
Diacetyl	*Saccharomyces lactis, Leuconostoc dextranicum*
Esters	
Geranyl acetate	*Ceratocystis virescens,C. variospora*
Ethyl butyrate and ethyl hexanoate	*Pseudomonas fragi*, *Streptococcus lactis*, *S. cremoris* *S. diacetylactis*, *L. casei*
Ethyl isovalerate	*Pseudomonas fragi*
2-Phenyl ethanol	*Erwinia carotovora*
Lactones	
α-Decalactone	*C. moniliformis*
t-Decalactone	*Sporobolomyces odorus*
s-Decalactone	*Saccharomyces cereviseae*, *Candida pseudotropicali*, *Sarcina lutea*
L-Menthol from menthyl esters	*Saccharomyces*, *Bacillus*, *Trichoderma*, *Candida*, *Rhizopus*
Pyrazynes	
2-Methoxy-3-isopropyl pyrazine	*Pseudomonas perolenes*, *Streptomyces*, *Streptococcus lactis*
Tetramethyl pyrazine	*B. subtilis*, *Corynebacterium glutanicum*
Terpenes	
Linalool	*Ceratocystic variospora*, *C. moniliformis*, *Phellinus igniarus*, *Kluveromyces lactis*, *Asocoidea, Lentinus lepidus*
Citronellol	*Ceratocystic variospora*, *C. moniliformis*, *K. lactis*, *Trametes odorate*
Geranyl acetate	*Ceratocystic virescens*
Citronemmyl acetate	*Ceratocystic coarulescens*

Source: Modified from Berger (1995) and Singhal and Kulkarni (1999).

Two metabolic pathways can be followed in the formation of esters: alcoholysis of acyl-CoA compounds and the direct esterification of an organic acid. Yeasts follow predominantly the first pathway, and filamentous fungi and bacteria prefer the second (Welsh and others 1989).

Some ramified amino acids are important precursors of flavor compounds and are related to fruit maturation. The initial reaction is called the enzymatic Strecker degradation. Several microorganisms including yeasts and bacteria such as *Streptococcus lactis* can modify the majority of the amino acid structures. Even if alcohols are related to fruit maturation, esters have a dominant role. Ethyl acetate comprises, with other compounds, banana flavor. 2-Methyl-ethyl-butyrate has a great impact on characteristic apple flavor (Fennema 1993).

Janssens and others (1987) found and quantified the fruity banana flavor produced by the yeast *Hansenula mrakii* and by the fungus *Geotrichum penicilliatum* in submerged fermentation using a synthetic medium. In the study using the yeast it was concluded that the fruity aroma occurred due to the biosynthesis of esters and alcohols. Seventeen compounds were identified in concentrations greater than 50 μL/L, including ethanol, ethyl acetate, isobutanol, ethyl propionate, isobutyl acetate, and isopenthyl acetate. Alcohols were formed in the exponential growth phase, but the esters were formed in the stationary phase. Ethyl acetate was the main product. In both studies, some precursors of fruity esters were added, such as vanillin, leucine, isoleucine, and phenylalanine. Thirty-three compounds were identified in concentrations greater than 50 μL/L: ethanol, ethyl acetate, ethyl propionate, and others. Ethyl acetate was produced in the highest concentrations (9924.1 μL/L).

Inoue and others (1994) reported the tolerance of *Hansenula mrakii* to ethyl acetate, which can be used as a sole carbon source. In this study, the esters formed during the production of sake by *Hansenula mrakii* and *Saccharomyces cerevisae*, were compared. Ethyl acetate, isobutyl acetate, and isoamyl acetate were preferably formed, determining the beverage quality. The formation of these compounds was catalyzed by the enzyme acetyl transferase from isoamylic acid and acetyl CoA.

Ethyl acetate, ethanol, acetic acid, and acetaldehyde were also produced by submerged fermentation and identified in the glucose metabolism of *Hansenula anomala*. In this case, the production of ethyl acetate was recognized as an aerobic process (Davies and others 1951).

Strains of *Ceratocystis* were also identified as ester producers. Lanza and others (1976) studied the production of acetates with different carbon (glucose, galactose, and glycerol) and nitrogen (urea and leucine) sources. They concluded that the type of flavor compounds produced depended on both sources (carbon and nitrogen), which is different for other microorganisms such as *Trichoderma viride*.

Collins and Morgan (1961) identified esters synthesized by different species of *Ceratocystis* (*C. moliniformis*, *C. major*, *C. coerulescens*, and *C. fimbriata*) during submerged fermentation in a dextrose potato medium. Ethyl acetate and ethanol were found in higher concentrations, except for *C. fimbriata*, which had isobutanol as the main compound. A strong banana flavor was detected when using dextrose and urea due to the presence of isoamyl acetate, which was also identified in leucine- or isoleucine-based media. For the combination galactose–urea, the main flavor was citric due to the formation of terpenes.

The yeast *Kluyveromyces marxianus* produced some compounds with characteristic fruity flavor, with ethyl acetate found in higher concentrations in solid-state fermentation

of different agro-industrial residues such as cassava bagasse (Medeiros and others 2000, 2001).

Banana flavor has also been identified in some plants when microorganisms (*Erwinia caratovora* ssp atroseptica) infected them. Spinnler and Dijian (1991) identified the volatiles formed in a synthetic medium, similar to the ones in the infected plant. From 13 isolated microorganisms with capacity to produce esters and alcohols, several compounds were identified; including ethyl acetate, 2-methyl-1-propanol, and propyl acetate. In this study, different media were tested with different nitrogen and carbon sources. Better results were found with glucose, fructose, sucrose, and asparagine. The addition of leucine led to the production of isoamyl acetate, corresponding to the degradation of the amino acid following the Erlich route.

Beiju is made of naturally fermented cassava, with fruity characteristics. It was used by the indians in Maranhão, North of Brazil, in order to produce a typical alcoholic beverage, *tiquira*. The microbiological population was identified and quantified by Park and others (1982), with counts between 6×10^5 and 1.9×10^6 CFU, as being predominantly *Aspergillus niger* and *Pecylomyces* sp.

Yoshizawa and others (1988) identified some volatiles produced by a strain of *Neurospora*, isolated by Park and others (1982), in submerged fermentation. These include ethanol, ethyl acetate, isoamyl acetate, ethyl hexanoate, and acetoine.

9.3.2.2 Aldehydes. Aliphatic, aromatic, and terpenoid aldehydes are important contributors to the flavor of fermented dairy products. They are synthesized by microorganisms as intermediates in the formation of alcohols from keto acids. An example is the bioconversion of ethanol to acetaldehyde by *Candida utilis.*

Flavor production using immobilized lipase from the yeast *Candida cylindracea* in a nonaqueous system has been studied for producing a broad range of esters including ethyl butyrate, isoamyl acetate, and isobutyl acetate. Ethyl butyrate has a pineapple–banana flavor, which has a large market demand, and sells at a price of US$150/kg upwards. This process has shown a great stability of the enzyme (more than a month) if kept hydrated intermittently (Singhal and Kulkarni 1999).

9.3.2.3 Alcohols. Alcohols do not contribute as a flavor component unless present in high concentration. They are formed as a primary metabolite from microorganisms' activity or due to the reduction of a carbonyl. Fusel alcohols can be formed from either carbohydrate or amino-acid metabolism and are the predominant volatiles of all fermented beverages, in addition to ethanol.

Different alcohols can be found in the culture of yeasts such as ethanol, propanol, isobutanol, and phenyl ethyl alcohol. In filamentous fungi it is possible to find methyl-3-buthanol, butanol, isobutanol, pentanol, hexanol, octanol-3, and phenyl ethanol from the metabolism of amino acids such as leucine, valine, isoleucine, and phenylalanine (Welsh and others 1989).

9.3.2.4 Carbonyls. Among the ketones, odd-numbered 2-alkanones from five to eleven carbons, along with free fatty acids and 2-alkanols, determine the flavor of *Penicillium*-ripened cheese and have received much attention. Bacteria such as *Aureobasidium*, yeasts, and higher fungi produce 2-alkanones, but only *Penicillium* has been used industrially.

9.3.2.5 Terpenes. Terpenes are the most important natural components of essential oils to be used as flavors. Microorganisms are able not only to synthesize but also to degrade or transform terpenes. Fungi are the main microorganisms responsible for terpene production, but bacteria are capable of synthesizing a few volatile terpenoids, such as geosmin and cadin-4-ene-1-ol (Berger 1995).

The synthesis of monoterpenes by *Ceratocystis variospora* has been studied by Collins and Halim (1972). Numerous other microorganisms are able to synthesize monoterpenes, among them *Ceratocystis moniliformis*, *Kluyveromyces lactis*, *Sporobolomyces odorus*, *Trametes odorata* and *Trichoderma viridae*.

Microbial bioconversion of terpenes has been studied by several authors. Monoterpenoid compounds like citronellal, citral, limonene, and menthol (acetates) can be biotransformed in citronellol, geranic acid, carveol and 1-menthol, respectively.

Valencene is a sesquiterpenoid available from orange oil and has little commercial use. Some bacteria are capable of transforming valencene to nootkatone, a main flavoring component of grapefruit.

From the economic point of view, the development of biotechnological processes for the production of terpenes is not viable due to the low yields obtained and the abundance of vegetable sources available. The real meaning of these studies is the understanding of the steps of the catabolism of terpenes.

9.3.2.6 Lactones. Lactones are associated with fruity, coconut, buttery, sweet, or nutty flavors. *Trichoderma viridae*, a soil fungus, generates a characteristic coconut flavor due to the production of 6-pentyl-2-pyrone. The main component of peach flavor, 4-decalactone, can be synthesized by *Sporobolomyces odorus. Aspergillus niger* can transform β-ionone into a complex mixture resembling tobacco flavor. Lactones make a significant contribution to the flavor of several fermented foods like dairy products and alcoholic beverages.

Some microorganisms such as *Ceratocystic moniliformis*, *Trichoderma viride*, *Sporobolomyces odorus*, and some species of *Candida* have been reported as lactone producers. However, the production is not very significant and has low yields (mg/L), except for the *in situ* production of lactones from dairy products.

Lee and Chou (1994) verified that the addition of 3% castor oil to the culture medium raised the production of lactones by *Sporobolomyces odorus*, with a yield of 8.62 mg/L.

Among lactones, 6-pentyl-α-pyrone (6-PP) presents the most interesting flavor properties. It is a molecule with a strong coconut flavor and is also present in the aroma of peaches and nectarines. The production of 6-PP by *Trichoderma harzanium* with sugar cane bagasse by solid-state fermentation was studied by Sarhy-Bagnon (1999) as an alternative for the production by submerged fermentation, giving a six fold raise in concentration.

9.3.2.7 Pyrazines. Pyrazines are typical flavor components of heated foodstuffs. They give the roasted or nutty flavors characteristic of roasted nuts, coffee, and cocoa beans, and baked and meat products. Microwave foods need the addition of pyrazines because they do not develop a characteristic nonenzymatic browning flavor during cooking. *Bacillus subtilis* was the first organism found to produce pyrazine. Pyrazines were also identified in cultures of *Septoria nodorum* and *Aspergillus parasiticus*.

9.3.3 Enzymatic Technology

Enzymatic processes that are used to obtain flavors can be described by the hydrolysis of some compounds without microbial growth. The majority of the enzymes used in food processing are hydrolases, such as amylases, proteases, pectinases, cellulases, pentonases, invertase, and lactase. They are used, for example, in cheesemaking (lipases, proteases), wine and juice production (pectinases), lactose reduction (lactase). Immobilization techniques, such as gel inclusion, microcapsules entrapment, and covalent or adsorptive binding onto solid supports has improved technical aspects such as handling, recycling, and long-term stability. Microbial enzymes have become an integrated part of processes in the food industry, So it is natural to see their use for the generation of flavor compounds (Whitaker 1991).

Filamentous fungi are capable of producing enzymes that are used to hydrolyze plant cell wall and liberate its content. However, the enzymatic extraction needs a thermal treatment, which sometimes can destroy or change flavor compounds.

Pectinase, cellulase, and hemicellulase of *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma* are enzymes more commonly used to increase extraction efficiency during fruit, vegetable, cereal, or juice processing (Armstrong and others 1989).

Lipases often show complex patterns after isoeletric focusing, but this heterogeneity is due to the varying degrees and positions of glycosilation of the protein core. Pre-pro-lipase and pro-peptides are now studied in detail by genetic engineering (Berger 1995).

Microbial hydrolases have been reported to improve the sensory quality of food by the synergistic action of mono-, oligo-, and poly-glucanases. Various carbohydrases have been purified and characterized, among them, β-glucosidase from *Aspergillus niger* and α-glucosidase, with maltase properties from banana pulp. Carbohydrases have contributed to the assessment of the identity and origin of plant products, to the understanding of changes during processing and maturation, and to the selection of flavor-rich cultivars. Stability and selectivity data will be decisive for sensory changes in a food and thus for the future application of new enzymes in food processing (Berger 1995). Smaller peptides and free amino acids, which are end products of various proteases, contribute to the non-volatile flavor fraction and act as precursors of volatiles.

Cheese treated with enzymes to enhance flavor, or a significant portion of the flavor profile, is considered to be enzyme-modified cheese (EMC). It provides the food manufacturer with a strong cheese note in a cost-effective, nutritious, and natural way (Moskowitz and Noelck 1987). Such EMCs are ideal in frozen cheese, because proteins from natural cheese tend to coagulate and produce a grainy texture, but the proteins in EMCs have been hydrolyzed to more soluble peptides and amino acids, overcoming these problems (Missel 1996).

EMC flavors available commercially include Cheddar, Muzzarela, Romano, Provolone, Feta, Parmesan, Blue, Gouda, Swiss, Emmental, Gruyere, Colby, and Brick. These cheese flavors have a wide range of applications in salad dressings, dips, soups, sauces, snacks, crisps, pasta products, cheese analogs, frozen foods, microwave meals, ready-made meals, canned foods, crackers, cake mixes, biscuits, quiches, gratins, cheese spreads, low-fat and no-fat cheese products, and cheese substitutes (Buhler 1996).

The basis of EMC technology is the use of specific enzymes acting at optimum conditions to produce typical cheese flavors from suitable substrates. These enzymes consist of proteinases, peptidases, lipases, and esterases. EMCs can be used in food recipes to fulfill several roles, for example, as the sole source of cheese flavor in a product, to

intensify an existing cheesy taste, or to give a specific cheese character to a more bland-tasting cheese product (Anon 1993). They have approximately 15 to 30 times the flavor intensity of natural cheese and are available as pastes or spray-dried powders (Freund 1995). The production of EMC is an important industrial activity, which has grown due to a greater demand for convenience foods, together with the health-related concerns regarding the amounts of fat, cholesterol, and cholesterol-producing saturated fat in traditional dairy products. EMC has been included in no-fat and low-fat products, replacing the functional and flavor characteristics of fats previously derived from natural cheese (Anon 1993; Freund 1995). The addition of EMC creates the desired flavor without increasing fat content. It can be added at levels of 0.1% (w/w) and contribute less than 0.07% fat or 2.28 calories per 100 g. Most new applications are targeted at texture and provide rich mellow tones, pleasant flavor-enhancing effects, fatty mouth-feel, flavor masking, rounding-off of sharp spicy notes, and harmonization of other flavor ingredients (Buhler 1996).

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10

Flavor Production by Solid and Liquid Fermentation

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10.1 Introduction 193
10.2 Fermentation Systems 194
10.2.1 Effect of Environmental Conditions on Flavor Production 194
10.2.1.1 Biological Factors 194
10.2.1.2 pH 194
10.2.1.3 Temperature 194
10.2.1.4 Nutrients 195
10.2.1.5 Water Activity 196
10.2.1.6 Aeration 196
10.2.2 Types of Fermentation Processes 196
10.2.2.1 Liquid Fermentation 196
10.2.2.2 Solid-State Fermentation 197
10.2.3 Bioreactors 198
10.3 Extraction of Flavor Compounds 200
References 202

10.1 INTRODUCTION

Microbial fermentation is a promising biotechnological technique for the production of natural flavors. Although many biotechnological processes have been reported, most have not yet been applied in industrial production. The major reason for this is the low yield. Microbial flavors are often present in low concentrations in fermentation broths,

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resulting in high costs for downstream processing. This is compensated by the price of the naturally produced compounds, which is 10 to 100 times higher than that of synthetic ones. It is estimated that around 100 flavor compounds are produced industrially by microbial fermentation (Janssens and others 1992).

The aim of current research is the development of low-cost processes with high yields. In order to achieve that, it is necessary to control the metabolic pathway and to develop alternative production techniques, such as the use of solid-state fermentation, immobilized cells, or genetically modified organisms.

10.2 FERMENTATION SYSTEMS

Any bioprocess is designed according to the microbial growth pattern and by the selection of the fermentation technique in which the desired product is produced by the microorganism. The bioprocess technique takes into account the physiological and morphological characteristics of the microorganisms and the properties of the products (Drawert 1987).

There are many factors that should be considered for the establishment of a fermentation process. These include the screening of suitable microorganism and substrate, optimization and adjustment of process parameters, design of the bioreactor, and downstreaming of the flavor compounds produced (Pandey 2003).

10.2.1 Effect of Environmental Conditions on Flavor Production

Physical, chemical, biological, physiological, and genetic factors can affect flavor production, among them pH, temperature, medium composition, inoculums' size, and strain selection.

10.2.1.1 Biological Factors. Desired microorganisms have to be present in the culture medium in sufficient amounts to carry out fermentation at an economical rate. In practice, it is achieved by applying a sterilization method, which eliminates undesirable microorganisms (Nigam and Singh 1999).

10.2.1.2 pH. Generally, the pH of the medium changes as a result of the microorganism's metabolism and growth. As carbohydrates are metabolized, organic acids are formed and the pH decreases significantly. Therefore, it is important to control the pH of the medium, as it affects microorganism growth, fermentation rates, and byproduct formation. Furthermore, in low-pH media there is no contamination (Pandey 1999).

Ceratocystis fimbriata cultivated on coffee husk achieved its best flavor yields at an initial pH of 6.0. Different medium pH resulted in lower levels of the fruity characteristic flavor (Soares and others 2000a; Medeiros 2003).

10.2.1.3 Temperature. Microbial growth and product formation are the result of many chemical reactions. The temperature influences them all. As microorganism growth and metabolism produce heat, cooling may be necessary to maintain the desired temperature. Temperature should be controlled, especially when volatile flavors are produced (Nigam and Singh 1999).

Optimal temperatures for growth and product formation are often different and have to be examined separately. It is important in particular for secondary metabolites, like many

flavor compounds. There is a need to determine the temperature at which growth rate is adequate and product formation is maximized. In the case of a secondary metabolite, it is interesting to maintain growth at low rates (Nigam and Singh 1999).

Sarhy-Bagnon (1999) studied the production of 6-pentyl-α-pyrone by the fungus *Trichoderma harzanium*, which has a characteristic coconut flavor. The best flavor production was reached at 25°C. However, 30°C was found to be the optimal temperature for growth.

Yeast metabolism makes an important contribution to beer flavor. Higher temperatures increase yeast metabolism, but the temperature will affect differently each biochemical reaction, changing the balance of flavor compounds. Bottom-fermented beer is produced at lower temperatures due to the fact that fermentation above 14°C results in a product with significantly poorer flavor and aroma (Smogrovicová and Dömény 1999).

10.2.1.4 Nutrients. Nutrients are substances used in the biosynthesis and energy production required for microbial growth and product formation. The nutrients may be classified as macronutrients (C, H, O, and N) and micronutrients (P, K, S, and Mg). The requirement for trace elements in the medium is a qualitative factor. In general, their concentration is arbitrary and they have to be present in sufficient amounts to avoid deficient growth of the microorganism (Pandey 1999).

Carbon Sources. The nature of the carbon source is another factor that affects flavor quality. This fact was verified by Hubball and Collins (1978) with cultures of *C. variospora* in the presence of many combinations of sugars and acids. When combining glucose, fructose, and arabinose with acetic acid, there is production of banana, strawberry, and lemon flavors, respectively.

Bramorski and others (1998a) investigated the possibility to improve the sensory properties of solid agricultural substrates through the synthesis of volatile compounds by the edible fungus *Rhizopus oryzae*. Cassava bagasse, soybean meal, and apple pomace were used in various proportions to study the influence of the C/N (carbon to nitrogen) ratio on the production of volatile metabolites. The volatile production was strongly dependent on the medium composition, as indicated in Table 10.1.

TABLE 10.1 Total Volatile Compounds Produced by *Rhizopus oryzae* at Different Solid Substrate Compositions.

Substrate (w/w)	C/N	Odor Detected	Total Volatile (μmol/L)
Cassava bagasse + soybean (8 : 2)	29.3	Mild, pleasant	13.5
Cassava bagasse + soybean (2 : 8)	10.8	Slightly ammoniacal	15.1
Cassava bagasse + soybean (5 : 5)	20.5	Mild, pleasant	23.0
Apple + Cassava bagasse + soybean (8 : 1 : 1)	35.6	None	38.2
Apple + Cassava bagasse + soybean (3 : 3 : 4)	18.0	Mild, pleasant	21.9

Nitrogen Sources. In general, the concentration of nitrogen compounds in the environment is low and, therefore, cells that grow fast in low N concentrations have an important competitive advantage.

Ceratocystis fimbriata is recognized as a fungus that can generate fruity flavors. Christen and others (1994) tested the influence of six nitrogen sources (leucine, urea, ammonium sulfate, asparagine, casein peptone, and fish hydrolyzate) on the nature and intensity of the flavors produced. They observed that leucine, urea, asparagine, and ammonium sulfate, to a lesser extent, were adequate for producing fruity notes. Leucine is known as a precursor of isoamyl alcohol and isoamyl acetate, which are major constituents of banana flavor. High amounts of metabolites were produced with those sources due to the maximum biomass concentrations.

Different nitrogen sources were tested for the production of coconut flavor by *Trichoderma viride* (Yong and others 1985). It was shown that a variation of the nitrogen source affected the quantity of 6-pentyl-α-pyrone produced, this being the lactone responsible for the characteristic flavor, but the flavor quality of the culture was the same for any nitrogen source.

10.2.1.5 Water Activity. In fermentation processes the water requirement is expressed in terms of water activity, which is the partial vapor pressure of water in the air in relation to the vapor pressure of pure water at the same temperature. Water is important to the microbial cell, not only because it represents about 80% of the cell's weight, but also as it is a reactant (as in hydrolysis reactions) and a product. In addition, water provides the most common environment for microbial growth. All living organisms need water for their growth and metabolism (Doelle 1985). The moisture content should be optimal. The moisture in solid-state fermentation exists in absorbed or associated form within the solid matrix, which can be more advantageous for growth because of the more efficient oxygen transfer process.

10.2.1.6 Aeration. In solid-state fermentation, the water content is quite low and the microorganism is almost in contact with air oxygen, unlike in the case of submerged fermentation. The low volume of water present in the media per unit mass of substrate can substantially reduce the volume of the fermentor without severely sacrificing yield. Aeration and mixing requirements may also be easily met (Raghavarao and others 2003). In submerged fermentation, the transfer of oxygen to microorganisms is the major difficulty (Durand 2003).

10.2.2 Types of Fermentation Processes

10.2.2.1 Liquid Fermentation. Most industrial fermentations are carried out in liquid media, also called submerged fermentation (SmF), in which microorganisms are dispersed into a nutrient liquid using a bioreactor. Brewing industries have used this kind of fermentation. The reactor, a stirred or nonstirred tank, could run as batch or continuous (Nigam and Singh 1999).

There are a number of flavors compounds produced by the liquid fermentation technique on an industrial scale. An example is the production of (R)-δ-dodecanolide, a lactone applied as a butter flavor in margarine. The bioconversion of 5-ketododecanoic acid takes place on a 30,000-L fermentor using baker's yeasts (Janssens and others 1992). The production of peach aroma (4-decalactone) involves the conversion of ricinoleic acid, an oil obtained from the

seeds of *Ricinus communis*, known as castor oil. The yeast *Yarrowia lipolytica* converts the oil into 4-hydroxydecanoic acid, which is easily lactonized. A yield of 6 g/L can be reached (Janssens and others 1992). *Clostridium butyricum* produces butyric acid from glucose in concentrations that reach 1–2% in the fermentation broth. Butyric acid can be applied as a natural cheese flavor and its esterification with ethanol gives rise to ethyl butyrate, an important fruity flavor (Janssens and others 1992).

Another example of the liquid fermentation process is the production of vanillin, which is widely used as a flavoring agent in a wide range of foods and fragrances. The use of biotechnological methods involving fungi to produce vanillin is being developed (Lomascolo and others 1999). Ferulic acid, a constituent of various grasses and crops, is a product of the microbial oxidation of lignin, particularly by white-rot basidiomycetes. This compound has been used as a direct precursor in bioconversions to vanillin. However, its recovery as a pure compound is difficult. Besides, low conversion yields are obtained due to numerous side reactions. An alternative for the production of vanillin is the use of solid-state fermentation. Some agro-industrial residues, which often contain ferulic acid in cell walls, may be used as substrates (Feron and others 1996).

10.2.2.2 Solid-State Fermentation. Solid-state fermentation (SSF) can be defined as a method to cultivate microbial cells in which organisms are grown on solid substrates or supports in the absence of free water. The moisture content is just sufficient to assure growth and metabolism of the microorganism (Pandey and others 2001).

Due to a low level of water activity, SSF processes have advantages in comparison with submerged liquid fermentation. These include high volumetric productivity, low capital investment and energy requirements, the possibility of using alternative substrates (e.g., solid residues) without pretreatments, less wastewater output, and easier product recovery. However, there are some disadvantages, such as the difficulty in removing excessive heat generated, problems in fermentation control, sterilization, and contamination (Feron and others 1996). They are more difficult to control due to the lack of adequate sensors and efficient solid handling techniques, especially for continuous operations. Furthermore, SSF processes are slower than liquid fermentations due to the additional barrier from the bulk solid (Raghavarao and others 2003).

Agro-industrial residues such as sugarcane and cassava bagasse, apple pomace, and coffee husk and pulp have been used efficiently in several bioprocesses. Not only does the application of agro-industrial residues provide alternative substrates for SSF, but it also helps in solving pollution problems (Soccol and Vandenberghe 2003).

Cassava and sugarcane bagasse, apple pomace, giant palm bran, and coffee husk have been used as substrates for flavor production in SSF (Bramorski and others 1998a, 1998b; Soares and others 2000a, 2000b; Medeiros and others 2000, 2001). Fruity flavors were detected in cultures of *Ceratocystis fimbriata* using coffee husk as substrate. In Table 10.2, Soares and others (2000a) have shown results of SSF under different experimental conditions. It was found that the flavor detected in the headspace of the culture depended on the amount of glucose added to the medium. Increased levels of glucose decreased flavor intensity. According to the authors, glucose concentration seemed to have a direct effect on metabolic pathways and thus on the nature of the volatile compounds produced (Fig. 10.1*a*). Among the compounds produced, ethanol and ethyl acetate were the most abundant.

Soares and others (2000a) investigated the influence of the addition of salt solution, soybean oil, and leucine on flavor production. According to Figure 10.1*b*, salt solution,

TABLE 10.2 Sensory Evaluation of the Headspace from *Ceratocystis fimbriata* Grown on Coffee Husk in Different Conditions.

Glucose (g/100 g)	Addition	Flavor and Intensity*	Total Volatile (mmol/L.g)
20	—	Pineapple +++	6.58
35	—	Fruity, pineapple +++	5.24
46	—	Banana, sweet +	1.13
35	Salt solution	Banana +	2.71
35	Soybean oil	Banana ++	5.7
35	Leucine	Banana +++	8.29

*Intensity: +++: strong; ++: medium; +: weak.

which was added to determine if the substrate lacked some minerals, caused a strong negative effect on flavor production (run 35.S). Addition of soybean oil (run 35.O) did not affect flavor production when compared to control (run 35).

10.2.3 Bioreactors

A suitable bioreactor design includes heat and mass transfer effects and easy diffusion and extraction of metabolites. Maximization of the rate of formation and yield of product within the bioreactor is a key part of optimizing the production process. In contrast to SmF systems, the development of an SSF bioreactor has some problems, such as poor mixing and heat transfer characteristics and material handling (Pandey 2003).

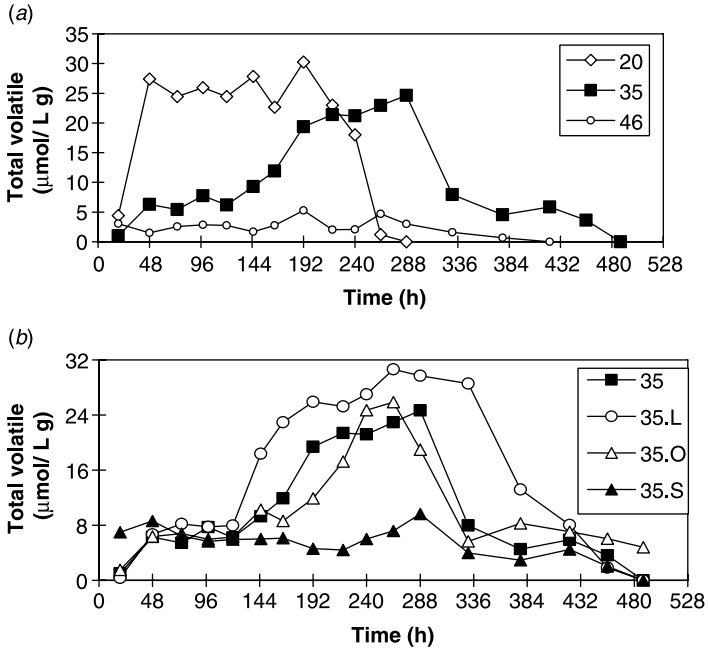

Figure 10.1 *Dynamics of total volatile compounds in the culture of* C. fimbriata *in coffee husk supplemented with (a) different glucose concentrations and (b) different compounds (L, leucine; O, soybean oil; S, salt solution).*

Most of the flavor-producing microorganisms are oxygen dependent. Therefore, a subdivision of the usual bioreactor is done according to the mode of gas supply. The oxygen can be supplied by using moving assemblies or pumps. Reactors with a turbulent layer are frequently used for yeasts and higher fungi, which can be propagated in submerged culture as well as in surface reactors (Drawert 1987).

A large number of patents and publications are available on how to design, operate, and scale-up SSF bioreactors. As SSF is more applicable for filamentous fungi, the process in most of the cases is aerobic (Pandey 2003). The main bioreactors used for SSF can be classified as tray, packed-bed, rotating drum, gas–solid fluidized-bed, stirred aerated bed, and rocking-drum bioreactors.

SSF processes can be operated in batch, fed-batch, or continuous modes, although batch processes are the most common. Some important aspects have to be considered during the construction of a bioreactor, among them the sensitivity of the substrate and/or the microorganism to the shear forces generated by agitation. Rotating-drum bioreactors operating at high rotation speeds damage the substrates and the hyphae of the fungus due to shear. Another aspect that occurs in the case of microbial degradable substrates is the shrinking of the substrate bed. This might result in an undesirable channeling of air through the gaps between the bed and the walls. This could be avoided by using an inert support or a natural substrate in which the solid structure is not attacked by the microorganism (Raghavarao and others 2003).

Some recent developments in automated bioreactors include operations such as the sterilization of the substrate, inoculation, control of fermentation conditions, extraction of the product from the substrate, and poststerilization of the substrate.

Medeiros and others (2001) produced flavor compounds with fruity characteristic in an SSF system. Cassava bagasse was used as the substrate and was inoculated with a GRAS (generally recognized as safe) strain of the yeast *Kluyveromyces marxianus*. The SSF was carried out by packing pre-inoculated solid medium (20 g cassava bagasse) into glass columns (4 cm diameter and 20 cm length). Figure 10.2 shows the schematic set-up of the fermentation system. The fermentation temperature was maintained at 28°C and the columns were connected with an air distributor. The authors studied the influence of different aeration rates on volatile production. It was found that ca. 7 μmol/L/g of esters were produced by the yeast at a low aeration rate (0.06 L/h/g).

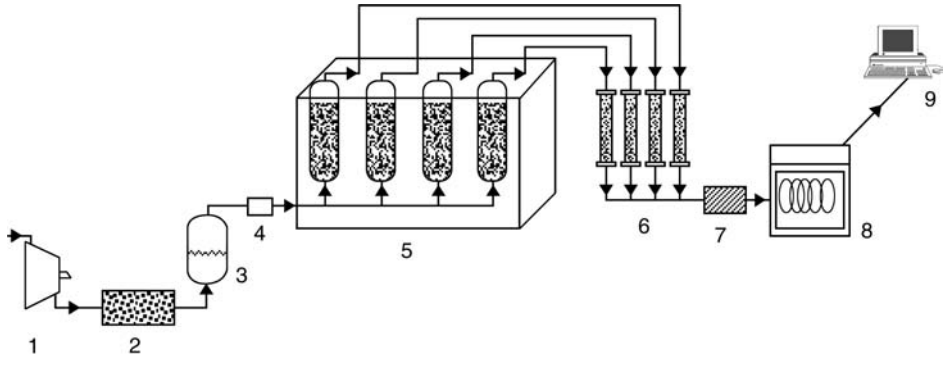

Figure 10.2 *Schematic set-up of packed-bed bioreactor system (1: air pump; 2: air filter; 3: air moisturizing unit; 4: air distributor; 5: bioreactor columns in water bath; 6: silica gel columns; 7: auto gas sampler; 8: gas chromatograph; 9: computer).*

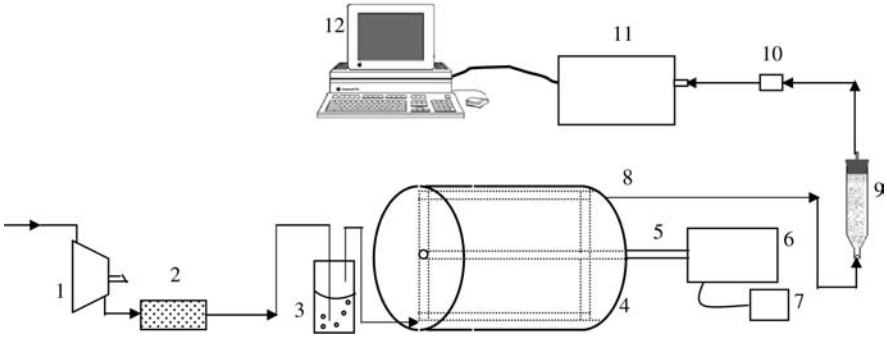

Figure 10.3 *Schematic set-up of a bioreactor system for flavor production by SSF (1: air pump; 2: air filter; 3: air moisturizing unit; 4: bioreactor; 5: paddle; 6: agitation motor; 7: rpm controller; 8: gas outlet; 9: silica gel columns; 10: auto gas sampler; 11: gas chromatograph; 12: computer).*

A stainless steel cylinder bioreactor (Fig. 10.3) connected with an air distributor was used to produce flavor compounds using *Ceratocystis fimbriata* (Medeiros 2003). Experiments were carried out using 3 kg of coffee husk as substrate. The volatile metabolites released by the culture were collected by connecting adsorbent tubes at the outlet of the bioreactor. The adsorbent tubes were made of glass and were packed with granular activated carbon (Ultraporous 6–8 mm, FBC Brazil); Tenax (Tenax TA, 60–80, Supelco); or Amberlite XAD-2 (Aldrich). The adsorbed volatile compounds were eluted with solvent and the concentrated solution of volatiles obtained was ready to be analyzed by gas chromatography.

10.3 EXTRACTION OF FLAVOR COMPOUNDS

Product recovery is often a difficult step in bioprocesses, especially for flavor compounds because of their volatility and low solubility. It is also necessary to keep the concentration of volatile compounds on the fermentation medium below a certain level due to their inhibitory effect in microbial growth. There are many on- and off-line technologies that can enable the extraction and concentration of flavor compounds (Bluemke and Schrader 2001).

To maximize the efficiency of the bioprocess, production of flavors should be maximized and the production of new cells minimized. In the case of cultures with a high cell density, integrated systems based on ultrafiltration and microfiltration techniques have been studied. Another technique is cellular immobilization. Senemaud (1988) immobilized cells of *C. moniliformis* in alginate beads to control the production of esters, alcohols, and monoterpenic alcohols.

As the toxicity of the hydrophobic flavor compounds leads to inhibitory effects on microorganisms, the efficiency of biosynthesis improves with the recovery of the compounds from the culture medium. One of the most used methods to remove organic compounds from fermentation media involves solvent extraction, separation on specific membranes, and adsorption on activated carbon and porous hydrophobic polymers. The two last methods have been used for the concentration of aroma compounds. Activated carbon has a high adsorption capacity which allows the use of small amounts of material; however, desorption is not always complete. Porous polymers commonly used include Tenax GC (Supelco), Porapak Q (Interchim); Chromosorb 105 (Altech Associated); and Amberlite XAD-2 and XAD-4 (Prolabo) (Souchon and others 1995).

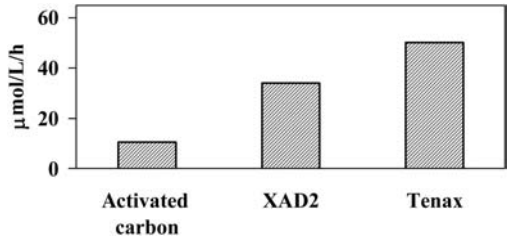

Figure 10.4 *Adsorption of volatile compounds produced by* C. fimbriata *cultivated on coffee husk using different adsorbent materials.*

There are numerous reports on the adsorption of flavor compounds. Sorption on activated carbon and porous hydrophobic polymers is a suitable method to extract and concentrate γ-decalactone from aqueous media. Solid sorbents were used in an on-line extraction of γ-decalactone during a bioconversion process (Souchon and others 1996). The sorbents tested were activated carbon and three porous polystyrene-type polymers [Porapak Q (Interchim); Chromosorb 105 (Altech Associated); and Amberlite XAD-2 and XAD-4 (Prolabo)]. *Sporidioolus salmonicolor* was cultivated on a fermentation broth. Adsorbents were added to the culture medium at 20 g/L and 30 g/L. γ-Decalactone was extracted from the adsorbents using hexane. The presence of adsorbents in the bioconversion medium allowed a very low concentration of the lactone in the liquid medium; as a consequence, it limited the toxicity of the flavor compound to the yeast. Carbon activated and porous resins (Amberlite XAD-2 and Tenax) were used to collect the flavor compounds released by the solid-state culture. The adsorbent columns were attached to the end of the bioreactor-type column fermentation. The advantage of Amberlite XAD-2 (Aldrich) and Tenax (Tenax TA, 60–80, Supelco) over activated carbon in collecting flavor compounds is shown in Figure 10.4 (Medeiros 2003). The hydrophobic character of the resins allows higher efficiency compared to activated carbon.

Spray-drying has been widely used for micro-encapsulation of volatile materials. The water is removed by vaporization, and substances that are more volatile than water, for example, organic compounds, are retained. Food flavors are highly volatile and may be easily lost during drying. The retention of volatiles in spray-drying can still be improved if a high concentration of dissolved solids builds up on the surface of drops early in the drying process (Ré 1998).

Flavor compounds produced in SSF by *C. fimbriata* were encapsulated in a blend of maltodextrin and arabic gum and the emulsion obtained was spray-dried. The powder was suspended in water in order to analyze whether micro-encapsulation was effective in the recovery of flavor compounds (Medeiros 2003). According to Table 10.3, the main compounds produced during fermentation were efficiently recovered.

TABLE 10.3 Flavor Compounds Obtained by Micro-encapsulation Using Spray-Dryer.

Compound	Concentration (μmol/L)
Acetaldehyde	0.34
Ethanol	0.05
Ethyl acetate	0.44

The advantage of flavor compounds obtained as a spray-dried powder is their stability. The flavor components do not have direct contact with the hot air, which prevents oxidation and losses by evaporation (Ré 1998). In order to prevent losses during drying, processing conditions should be optimized. It was estimated that only 34% of the total volatile produced during SSF is recovered by micro-encapsulation during fermentation.

Pervaporation is a membrane process consiststing of partial vaporization of a liquid mixture through a nonporous permselective membrane (Souchon and others 1995; Karlsson and Trägárdh 1996). The technique allows potential applications in the food industry: for wine or beer dealcoholization, juice concentration, and extraction of substances produced by microorganisms. Flavor compounds can often be enriched several hundred times using this technique (Karlson and Trägárdh 1994). Lamer and others (1996) used pervaporation to extract benzaldehyde produced by *Bjerkandera adusta* in a 7-L glass fermentor. A system for continuous extraction of benzaldehyde was used. The performance of the process was studied using different parameters.

In situ product removal is an interesting tool for overcoming inhibitory effects on a microorganism's growth and production of flavor compounds. Some benefits are minimization of the interaction's products-producing cells, smaller product losses during cultivation, and reduction of subsequent downstream processing steps. An integrated bioprocess consisting of a bioreactor and a downstream unit was set up by Bluemke and Schrader (2001). A pervaporation membrane module was interlinked with a bioreactor for production of flavor compounds by *Ceratocystis moniliformis*. The product concentration in the bioreactor decreased and microbial growth rates increased. The yield of flavor compounds produced was higher than for batch fermentation.

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11

Herbs, Spices, and Essential Oils

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11.1 Introduction 206
11.2 Definition 206
11.2.1 Spices 207
11.2.2 Herbs 208
11.2.2.1 Fresh Herbs 209
11.2.2.2 Aromatic Herbs 209
11.2.3 Essential Oils and Sapid Compounds 209
11.2.3.1 Essential Oils 209
11.2.3.2 Sapid Compounds 212
11.3 The Spice Trade 213
11.4 Quality Aspects for Herbs, Spices, and Related Extracts 215
11.4.1 Authenticity 215
11.4.2 Cleanliness 215
11.4.3 Ash Level 216
11.4.4 Volatile Oil (VO) 216
11.4.5 Moisture Content and Available Water 216
11.4.6 Bulk Density 216
11.4.7 Particle Size 216
11.4.8 Heavy Metals 216
11.4.9 Pathogens 217
11.4.10 Pesticide Residues 217

Reviewed by Tim Sonntag, VP of Technical Services, Wixon Inc., and Carl Sheeley, President of Fontarome Chemical, Inc.

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

11.4.11 Mycotoxins 217
11.4.12 Other Specific Quality Tests 217
11.5 Spices and Herbs in the Food Industry 217
11.5.1 Whole Spices 218
11.5.2 Spice Blends 219
11.5.3 Seasoning Blends 219
11.6 Conclusion 219
References 220

11.1 INTRODUCTION

All living creatures on this planet spend a lot of time and energy trying to survive. Finding food to sustain their biological needs surely represents a major aspect of their life. Human beings are not really that different, but because of their level of complexity they are, by far, the most prone to making feeding more than just a necessary biological part of their existence. Apart from a few exceptions, humans are the only species that can modify their food resources by using cooking, fermentation, and other presentation techniques. One main reason for this habit is the quest for a higher level of satisfaction, which takes them way beyond the simple need to absorb all the necessary nutrients to have their body work properly. Let us face it, humans have become experts in looking for pleasure and food has certainly, and has for eons, represented one of the few aspects of their lives that can generate that sensation. Humans can eat almost anything, and if you travel a little bit around this planet, you will realize what anything really means. For one, you have to take into consideration what type of nutrient is available at any given time and location, and also the benefit this brings to the survival aspect of all living creatures. Humans are very adaptable. Combined with the ability to change their environment, it is no surprise they have spread around Earth to the extent they have.

Changing natural raw ingredients most certainly dates as far back as the mastering of fire. This essential capability has triggered a drastic change in human feeding habits. One benefit of cooking is obviously the possibility to develop chemical compounds that are not present in the raw product as explained in other chapters of this book. The other important side effect of cooking is to make food safer. One can reasonably assume that using fire for cooking has also contributed to humans increasing their curiosity as to other ways to modify their food. For example, the use of controlled fermentation has brought us beer and wine, which can definitely increase our satisfaction level, as well as milk derivatives such as yogurt. Another way has been to add other natural ingredients to the primary food product so as to complement its performance towards the ultimate satisfaction of Mankind. In this category we can include salt and of course herbs and spices.

The following paragraphs will try to give some clarification about what can be considered a spice or herb and also why their usage is important to modify the taste of our food. I would also like to stress that the title of this chapter (Herbs, Spices, and Essential Oils) may bring some confusion; these are not totally independent sections. We will also discuss the Spice trade, from its humble origin to its modern highly sophisticated situation. We will also evaluate the benefit that such products can bring to our ever-changing food habits.

11.2 DEFINITION

When looking at dictionaries, the definition of spices usually appears vague, if not redundant. The general consensus would be to define spices as aromatic or pungent botanical

products used to flavor food. Add the definition of flavor as being a mixed sensation of smell and taste, and you may understand why any interpretation would be as good as the next one. The definition of taste is somehow left to the reader's imagination and usually confines it to the sensation caused by aromatic or pungent compounds in the mouth. There is a lot more to it and the different aspects of taste help to understand why and how we can use these vegetable substances to modify our food. Spices and herbs belong to the angiosperms, plants where the reproductive organs are concentrated in a flower and whose seeds are locked in a fruit. This family is divided between monocotyledons and dicotyledons and represents more than 230,000 species around the globe.

11.2.1 Spices

The Codex Classification, Section 028, gives a good baseline for our understanding: "Spices are dried aromatic seeds, buds, roots, bark, pods, berries or other fruits from a variety of plants, which are used in relatively small quantities as seasoning, flavoring, or imparting aroma in food. They are consumed in the dried form after being added to or sprinkled on foods". "Dried" and "small quantities" are clearly important words here, and the Codex obviously leaves the fresh herbs and some aromatic vegetables on the side. A good reason is that fresh products may be of limited and even uncertain use. I have also recently read a proposal by the Codex Committee on pesticide residues that is a little more restrictive. The definition does not include plants that have been traditionally regarded as vegetables or fruits. The use of the word "traditionally" is not as innocent as it may seem when related to the taste concept. However, the article proposed an interesting sorting of the different species depending on the general Codex guidelines.

We can all grow a few culinary herbs in our back yard, but it would certainly become a challenge to use them for international trade. Fresh products spoil quickly, both in term of safety and quality, and until reliable transportation and conservation modes became available all around the globe, it was pretty difficult to maintain fresh products in a palatable form, and therefore use them for trade. Fresh spices were then consumed locally around their native growing location, and the dried products were the only form used to be sent to other parts of the world that were showing an increasing urge for them.

Traditional spices, understood as commonly used, come from a wide array of plants as shown in Table 11.1. In our occidental culture, we also apply an exotic connotation to the plants we refer to as spices. They historically come from regions of the world that have been out of reach for centuries and their availability has been subject to secrecy and even myths. We should not forget that each spice will in fact present several, and sometimes hundreds of subspecies, so that the name refers in fact to all cultivars. Each specific cultivar presents variations that can translate into color, shape, aroma, or taste changes, from subtle to very significant differences. Each subspecies presents variations in what contributes the most to taste and aroma, that is, the level and composition of the essential oil or pungent compounds they may contain. All these aspects tend to complicate classification, but extend our understanding that such a simple word as "spice" covers in fact so many botanical varieties. Mother Nature really offers what seem unlimited palatable options to Mankind.

Spices generally originated in warm and humid climates, mainly India and South-east Asia, and their availability was therefore limited in the past to those parts of the world. They all started in being collected from the wild until it became possible to apply agricultural techniques, which would transform picking into something more productive, and

TABLE 11.1 Classification of Some Usual Spices and Herbs.

Family	Common Name	Part of Plant
	Angiosperms	
DICOTYLEDON		
Solanaceae	Chili, paprika, red pepper	Fruit
Pedaliaceae	Sesame	Seeds
Piperaceae	Pepper, long pepper	Berries
Myristicaceae	Nutmeg, mace	Aril
Lauraceae	Cinnamon, cassia	Barks
	Bay leaf, laurel	Leaf
Cruciferae	Mustard	Seeds
	Horse radish	Root
Myrtaceae	Clove	Buds
	Allspice	Berries
Lamiaceae	Mint, basil, oregano, rosemary, sage, thyme	Leaf, twig, terminal, shoot
Umbelliferae	Anise, caraway, coriander, cumin, fennel, coriander, celery	Seeds, fruit
	Chervil, dill, parsley	Leaf
MONOCOTYLEDON		
Liliaceae	Garlic, onion	Bulbs
	Chive	Leaf
Iridaceae	Saffron	Pistil
Zingiberaceae	Ginger, turmeric	Rhizomes
	Cardamom	Fruit, seed
Orchidaceae	Vanilla	Fruit (pod)

mass transportation. As long as the supply could meet the needs of a slow-growing population, collecting and limited trade was good enough. The extraordinary expansion of European countries that started at the end of the fifteenth century changed the situation dramatically.

Spices have been around Mankind long enough, mostly used in their dried form for trade, such that their definition may vary around the globe. Each spice may also be referred to under local names. This has contributed, in the past, to maintaining trade secrets. Like any classification, there are always borderline situations and it is not rare to come across arguments about whether or not a given product would (or would not) qualify as a spice, given the wide array of definitions. Garlic, onion, or shallots are a good example. Many of us think of them as vegetables. Very aromatic indeed, but do they really qualify as a spice? As fresh products probably not, but very large volumes are traded as dry, diced, or ground product, and I would say they do indeed bring a lot of flavor to any dish, so let's include them in the spice category.

11.2.2 Herbs

In this category, and because of the Codex definition, it is intuitive to group together all plants where leaves are used for their aromatic properties. Herbs can be used fresh in our own kitchen or at a restaurant, where their highly delicate aromatic character is best preserved, but the vast majority of the trade is based on dried herbs. Herbs mainly relate to the Lamiaceae and Umbelliferae families as shown in Table 11.1. Their

growth does not require the same type of hot and humid climate as do the spices in general, which explains why they have been known and used without interruption for thousands of years around the Mediterranean Sea. There is a general consensus that there are two main categories of herbs: fresh and aromatic.

11.2.2.1 Fresh Herbs. Fresh herbs are also referred to as culinary herbs. They have a high water content and their aromatic character is really at its best when fresh. Some herbs, like parsley, chervil, or chives, withstand freezing nicely when chopped (color and aroma), so we can extend their domestic use throughout the winter. However, their industrial usage remains limited to restaurants. If dried, they tend to lose their aroma and their taste quickly evolves to hay. Freeze-drying herbs allows a very good preservation of the original green color, and prevents mold, and their industrial use is limited to provide a pleasant visual aspect in seasonings. The aroma contribution is negligible.

11.2.2.2 Aromatic Herbs. Herbs such as thyme, oregano, marjoram, rosemary, or laurel offer a more attractive industrial usage because of a much lower water content and higher aromatic compound level. The drying process maintains the aromatic character a lot better than what can be achieved with the fresh herbs, making them valuable trade goods. They also contain a wide range of functional compounds such as antioxidants, which has energized their value in the past few years.

11.2.3 Essential Oils and Sapid Compounds

Modern botanical studies and analytical techniques have helped us identify and classify species that have been known for thousands of years. We are also in a better position to understand what gives each spice or herb its character because we can identify their molecular constituents. Their properties have been known for ages as food or medicinal additives. They can be separated into two main groups: essential oils and sapid compounds.

11.2.3.1 Essential Oils. Spices and herbs are not the only botanical species that contain an essential oil (EO). The term "oil" is also commonly used in the flavor industry and should not be mistaken with just vegetable oil (triglycerides). Every plant produces some, but it may not always be of real value; either the quantitative level is too low to be beneficial in a food product, or the overall sensation it produces is not considered appealing by humans. It some cases, it can even be somehow toxic due to the presence of certain chemicals. Rue oil, for example, contains several aliphatic ketones. It has been known for centuries to be a rather effective abortive when the leaves were used in decoctions provided by medicine men or sorcerers. I should add that the frequency of use of rue oil in food flavoring is close to nothing.

Essential oils contain a very wide range of chemicals created as byproducts by plant cells during their regular photosynthesis and respiratory cycles. These molecules have a high vapor pressure that allows them to be considered as volatile at atmospheric pressure and room temperature, in the sense they can be detected by olfaction, which usually represents our first contact with the spice and is a very important part of our relation to food. Their influence on our perception of taste is also critical. Essential oils also have a significant antimicrobial activity; this is an additional benefit of using spices in food. Without any knowledge of the mechanisms, our ancestors had discovered this interesting property and used spices to preserve delicate food, along with salt. The composition of each

essential oil is characteristic of a plant, and their analysis by modern analytical chemistry equipment, such as gas chromatography and mass spectrometry, reveals the presence of the following components:

- Terpenes, general formula $C_{10}H_{16}$, such as limonene, α and β pinene, myrcene;
- Sesquiterpenes, general formula $C_{15}H_{24}$; such as β-caryophyllene, curcumene;
- Aldehydes (cinnamaldehyde);
- Alcohols (linalool, terpineols, eugenol, menthol);
- Esters (linalyl and terpenyl acetate);
- Ketones;
- O-ethers and S-ethers;
- carboxylic acids.

This list is by no means exhaustive, as continuous researches identify new molecules almost every day. The relative proportions of such molecular compounds play a major role in the final aromatic character of an oil. However, it is not only the proportion that is critical – the molecular conformation also plays a role, for example, as in isomers like estragole and anethole (Figs 11.1 and 11.2). Both have an anise-like aroma, but the overall taste is somehow vaguely different. Estragole is present in tarragon or bitter fennel oils and anethole in anise or sweet fennel. The difference can also be based on the stereo conformation, as in the case of carvone (Fig. 11.3). The D-carvone is the main component of caraway or dill oil, and the L-carvone may represent 80% of spearmint oil. There is no doubt that this difference would be clearly identified by aroma and taste. We can relate these changes to the influence of stereochemistry in pharmaceutical molecule activities.

It is important to remember that the composition of an essential oil varies between subspecies and is widely dependent on the plant phenotype, geographical origin, climatic

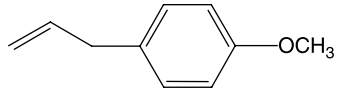

Figure 11.1 *Estragole.*

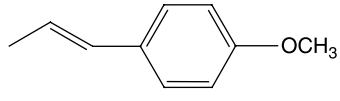

Figure 11.2 *Anethole.*

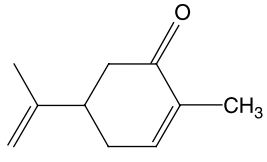

Figure 11.3 *Carvone.*

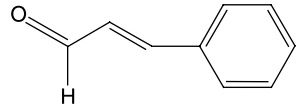

Figure 11.4 *Cinnamaldehyde.*

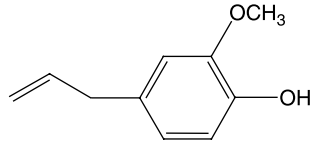

Figure 11.5 *Eugenol.*

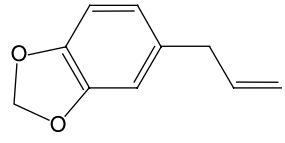

Figure 11.6 *Safrole.*

conditions, and even time of harvest. The composition may also vary between parts of the same plant, which makes some spices so valuable. The bark of the cinnamon tree produces an oil rich in cinnamic aldehyde, and the oil extracted from the leaves has eugenol as a main constituent (Figs 11.4 and 11.5).

The nutmeg–mace case represents the ultimate variation, because mace is the external envelope of the nutmeg fruit. Safrole (Fig. 11.6) is a major constituent of mace oil, but nutmeg oil has very little of it. Safrole is now considered carcinogenic and nutmeg fruits must be stripped of their external layer for trade. Safrole is also a major component of the sassafras root oil once used to make root beer or widely used in Cajun dishes.

The quantitative level of oil may vary from a fraction of a percent (herbs) to several percent (clove buds). It is therefore understandable why our perception would be affected so much. For more information on oil compositions, I cannot recommend enough that students and food chemists alike refer to what some of us call the "Dr Brian Lawrence's bible", in reality the best published review on essential oils composition (Lawrence 1979, 1981, 1989, 1993, 1995, 2003). There is no doubt in my mind it is a "must have" reference book for any chemist in the food and flavor industry.

Essential oils are not only consumed as part of the plant, but they are also extracted to be used in aromatic composition because this is where the taste and aroma is concentrated. Oils are then used to supplement low-quality natural products, or bring a typical character to a seasoning blend where the use of the straight spice or herb may represent a composition challenge. The following oil extraction methods have traditionally been used:

- Steam distillation (may induce oxidation) followed by decantation;
- Maceration in vegetable oil (old but simple technique that can still be used in the kitchen);
- Cold press (a must for citrus and any highly oxidation-sensitive oil).

11.2.3.2 Sapid Compounds. Apart from the volatile essential oil, plants, and more specifically spices, also generate some other highly interesting molecules. These are not present in the essential oil and because of their low vapor pressure we do not detect then by olfaction, but their importance on the taste is by no means negligible. The main attractions are the pungent compounds. Would black pepper be of any interest without piperine (Fig. 11.7)? And why would we use chili pepper if it was not for the presence of capsaicin (Fig. 11.8)?

Although these sapid compounds are present in the whole spice, the industry has rapidly developed specific extracts that concentrate those molecules in a form that facilitates their dispersion in a food product. As we discussed for the essential oil, we will not forget to mention the oleo-resins (commonly referred to as OR). Historically they have been obtained using solvent extraction of spices or herbs. The main solvent remains ethanol, but in some instances, acetone, ethyl acetate, or water may be of assistance. The solvent extraction concentrates the spice or herb aromatic compounds, both volatile and sapid. The final extract is usually stabilized with some oils (mono- and diglycerides) and is also standardized to a volatile oil (VO) content using the related essential oil. As an example, a commercial black pepper OR contains 40–42% piperine and has a minimum 20% VO content.

Another interesting category of sapid compounds contains the functional molecules. Their application in food has been more recent than it has been for pharmaceutical purposes. A very interesting category is the natural antioxidant, generally found in herbs such as sage, oregano, or rosemary. Their recent application to food is becoming a craze as consumers see a health benefit in moving away from more traditional (artificial) solutions. Their industrial importance also relies in the labeling benefit for food manufacturers, because they fall under the natural extract section. There is no doubt that more research will unveil other functional molecules that will represent future benefits for food.

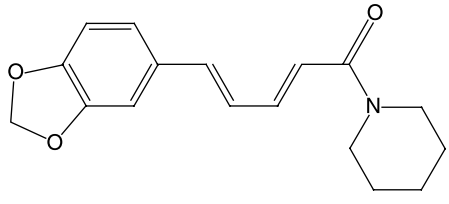

Figure 11.7 *Piperine.*

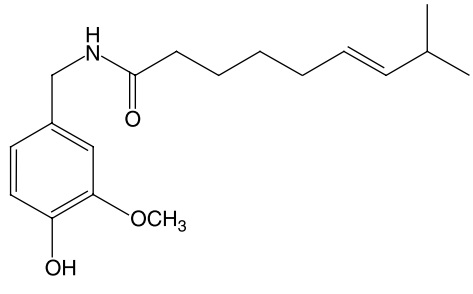

Figure 11.8 *Capsaicin.*

The knowledge of the chemical compositions of spices or herbs is very helpful for the food technologist to combine products and create appealing products. It also helps to understand the origin of a spice so as to maintain the authenticity and the quality of supplies, which in time will ensure the consistency of our food products.

11.3 THE SPICE TRADE

Spices and herbs have been available to humans for a very long time. Some recent archeological findings in the Swiss Jura mountains have unearthed fennel seeds dating back to the Neolithic age (5000 B.C.). Obviously, Homo sapiens tried whatever was available around them, and started to develop food habits. No-one will ever know how much deception or pain these attempts may have produced, and our knowledge will probably remain very vague. Apart from inhabitants fortunate enough to live in subtropical regions where spices grew naturally, others would yet have to discover them. For the European populations, such discovery would bring interest, then greed and the urge to conquer new territories.

As humans settled and started civilizations, their ability and urge to discover led them to trade. As time went by, trade routes emerged, expanded, and brought new knowledge, techniques, and products. Spices quickly became one form of goods that was highly prized. In ancient Egypt, spices such as cinnamon, anise, and cumin were used to embalm the dead. No doubt the essential oil they contain performed well to protect the tissues from decaying by stopping microbiological growth. In addition, their aroma surely helped cover the stench that probably surrounded the priests during the ceremony, and, in turn, spices received some kind of spiritual attribute, which definitely conferred them a special place in society: high value. Trade was made through Arabic merchants that had connections all the way to India and islands that are now Malaysia and Indonesia, and they maintained such a high level of secrecy and mythology that nobody really challenged them. In the 5th century B.C., the Greek historian Herodotus relates stories he gathered from Arabic merchants of large birds that jealously kept the spices on top of high mountains. The cover up worked so well that it kept people away and preserved the Arabic monopoly. Supplies had to come by land, using caravans that would take months of travel, through dangerous areas so that no-one else had the real capability to dominate the trade in the long term.

By the mid-4th century B.C., Alexander the Great would send expeditions east. I doubt this was just for glory, but also for an attempt to control what was an already lucrative business. Greeks were known as fierce merchants and their domination over the eastern part of the Mediterranean Sea was already complete. His armies reached the Indus River and the control of the spice trade was within grasp. Unfortunately, he died young and his efforts were abandoned quickly. In those days, and for centuries to come, such imperialistic efforts remained too dependent on individuals to translate into long-lasting political or economic systems.

Kingdoms evolved to empires and armies pushed their conquests ever further. Then the Romans took over the entire Mediterranean region and, by the end of the third century A.D., their Empire extended so far east it had nearly reached India. Trade routes were solid and supplies arrived to Rome where the rich population could enjoy a very wide array of foreign goods. Spices such as cinnamon, clove, pepper, or cardamom were plentiful for the few families that ruled the Empire, and their use in food, beverages, and

toiletries reached levels that would make us quiver. There are some documents, books or plays, that describe some "delicacies", and it would be quite an experience to try them today. No place for delicate combinations, but a rather constant quest to surprise and stun guests. Banquets were not so much a time for feeding and partying than a clear statement of wealth and power. As spices were expensive, they ideally served that purpose.

The demise of the Roman Empire by the fifth century A.D. would almost bring Western civilization to a stop and draw a curtain between the two sides of the Eurasian continent. Western European kingdoms would lose almost all their trade routes, apart from those maintained by the cities of Venice and Genoa in Italy; navigation and exploration would become a definite hazard when not considered a heresy. This period is sometimes called the Dark Age of Western civilization. It will last almost 900 years. The cost of spices, along with other imported goods, would skyrocket and become a luxury enjoyed by the wealthiest caste of ruling feudal societies. When in 1194 the king of Scotland paid a visit to his fellow monarch Richard I of England, he received, among other proofs of hospitality, daily allotments of 2 pounds of pepper and 4 pounds of cinnamon, obviously more than one person could consume. Spices had a ceremonial as well as a culinary function in the Middle Ages; the two were closely connected. Besides being used in food, spices were presented as gifts, like jewels, and collected like precious objects.

At the end of the fifteenth century, pushed by the Renaissance new ideas and awareness of some fragmented stories from the ancient days and the almost mythical Polo journey to China (1271–1295), a few adventurers were to force Europe to emerge from its long sleep. As we all know, Christopher Columbus sailed west to reach China as to avoid the Arabic route and found a new world instead. Among other things, his followers would discover "red pepper", a brand new pungent plant. Those daring men travelled to find new lands, gold, gems, new souls to be converted, but the desire to re-open and control the old spice trade routes must have played a significant role. After all, those expeditions had to be funded somehow. Political and spiritual blessing from kings was necessary, but in the end the money came from merchants and bankers, who had achieved an undisputed economical if not political strength by then.

When, in May 1498, the Portuguese Vasco de Gama set foot in the Indian city of Calicut (known today as Kozhikode), on the Oman sea, the long awaited time to control the spice trade had arrived. Western civilization was now in a better shape to maintain that control because of its new naval capabilities. It would not take long to erase the Arabic domination and develop all necessary economical, technological, and military tools to maintain that trade. Europe was to enjoy those goods so much that it would once again be a symbol of power. The spice trade would shape European countries and is certainly the point of origin for guilds, companies, and our modern capitalist system. It would also result in fierce competition between European countries. Portugal, until it was annexed by Spain in 1580, would dominate during the fifteenth and sixteenth centuries, and then the Dutch would take over during the seventeenth century. Naval domination took turns and whoever had the best battleships would surely win the domination. During the eighteenth and nineteenth centuries, England and France would fight over the southeast and Caribbean worlds alike, until spice seeds and plants were exported and cultivated all around the world.

The twentieth century would witness the creation of organizations such as ASTA (American Spice Trade Association) and ESA (European Spice Association) to regulate (and therefore set up necessary quality standards) a trade that represents hundreds of millions of dollars. Accurate numbers are difficult to obtain as there is still a great deal

of secrecy about costing. To this day, black pepper remains the king of spices and represents a good third of the international spice trade. In spite of multiple growing regions, the best quality remains its Indian ancestral location. Spices have now lost a large part of their traditional luxury or magical connotations and have become more of a commodity. As such, their price can vary dramatically, depending on climatic conditions, political turmoil, or just speculations from brokers or producers, and it takes a lot of experience and networking for any purchasing agent to keep up with any news that may affect pricing and availability. There are opportunities to obtain good deals, but also to end up empty-handed and have to pay premium dollars to get supplies in and on time, and you still may receive substandard quality.

11.4 QUALITY ASPECTS FOR HERBS, SPICES, AND RELATED EXTRACTS

An old saying states that you always get what you pay for, but you still want to make sure you really get what you have ordered. Within the spice industry, a close relationship between purchasing and their quality departments, brokers and local producers of spices have become the norm. What is at stake here is building trust between all parties. There have been, even recently, amazing stories of grossly adulterated shipments and malevolence and greed were not always out of the equation. The different trades associations have slowly developed rules to maintain quality and make sure that the final consumer is protected.

11.4.1 Authenticity

Because there are so many variants of each spice, the botanical subspecies must be understood by all parties, and specifications should clearly reflect them. Spices, herbs, and essential oils may be contaminated, either by accident or through malevolence. Adulteration has plagued this industry probably for as long as trade has been around. It is the chemist's duty to make sure the supplies meet specifications. To illustrate the possible mishaps we can face sometimes, let me relate the incident we had a few years back when one of our seasoning blends containing fennel seeds was rejected by a customer. The rejection was motivated by the president of that company, who had found their finished meat product did "not taste as it used to be". This gentleman had been in charge for decades and maybe some people would have argued about his tasting capabilities not being at their best by then. The problem was investigated, however, and we discovered the old gentleman was right after all. There had been a lot of fennel seeds that had contained a different variety of fennel; the one we had purchased was to contain estragole as the major ingredient in the oil, and the other one had anethole instead. Both were fennel seeds, but from different species. The difference was not dramatic, but it was noticeable. Different techniques from GC/MS to SNIF/NMR (site specific isotopic abundance) allow us to detect anomalies and adulteration.

11.4.2 Cleanliness

This measures the amount of extraneous matter, from dirt and wood to metal and insect fragments or rodent excreta. Cleaning works best on the whole product (before any grinding) and different methods have been put in place at the production level to eliminate

those contaminants, for example, air separators, destoners, indent and spiral separators. Control methods are either visual, under a microscope, or using screens on ground products to validate the cleanliness. This aspect has become a major concern worldwide as our concerns about food safety have increased dramatically since September 2001. Trade associations allow a certain amount of extraneous matter (small stones for example) and I am not sure this will not need to be revisited soon. Food manufacturers have improved their detection equipment, or acquired some (X-ray detectors), and the spice industry will have to react soon because the trend will be to have zero tolerance, not so much because of safety, but rather because of quality issues in direct connection with consumer feelings.

11.4.3 Ash Level

This measures the level of mineral residues after burning all carbon-based material at a usual 550°C temperature. It helps prove the effectiveness of the cleaning steps.

11.4.4 Volatile Oil (VO)

This test will determine the good organoleptic quality of the product and prevent accepting old or previously spent material. The oil is separated from the ground spice sample by steam distillation and read off in a volume proportional to the mass of the product under test. Each spice or herb has its own acceptable VO range.

11.4.5 Moisture Content and Available Water

This is an important measure as it directly pertains to the overall cost of a shipment. A 12% maximum value is usually accepted. A level of available water (Aw) of 0.6 has also been used in recent years to define a safe level under which microbial activity would be contained.

11.4.6 Bulk Density

This would mostly affect filling retail containers and applies to chopped, crushed, or ground products. This determination is fundamental when dealing with packaging design.

11.4.7 Particle Size

Most spices and herbs are usually ground to provide better dispersion in the final food product, which in turn helps the dispersion of the aromatic compounds. The particle size specification is maintained by means of metal screens (sieves). The aperture is characterized in microns or millimeters. The older method or mesh size (still widely used in the United States) relates to the number of holes per inch. As an example, a 25 mesh U.S. is equivalent to a 500 micron aperture. Conversion tables have been developed.

11.4.8 Heavy Metals

Spices, like any other plant material, contain heavy metals and their levels would usually meet the usual international or national standards for food products. Given the relatively low level of daily consumption, the health risk remains limited.

11.4.9 Pathogens

Depending on the treatment they undergo at the original production site, and the nature of the spice itself, pathogen contamination may happen. Spices and herbs have traditionally been dried outside, on the ground, and it is impossible to prevent contamination. Although Good Manufacturing Procedures (GMPs) tend to be implemented everywhere, a complete guaranty is impossible. Counts of over 1,000,000 (TPC) are not rare on some spices, and it is believed that 10% of consignments may contain such pathogens as Salmonella. Spices and herbs may then need to be treated. Different methods have been used, from ethylene oxide (ETO) fumigation to more modern irradiation or steam treatment. Ethylene oxide has now been banned because of potentially harmful residue concerns, and in some countries irradiation is not possible, or needs to be added on the label. Although irradiation is very efficient, in most cases, pressure from the consumer and a lack of sufficient technical skills may tend to limit its usage. Noncondensing steam has also been introduced in the past few years, and I have recently seen a unit in Italy. Its effectiveness is certain; it works in about 20 minutes and does not require the level of protection needed for irradiation. However, it does not really work well on fine ground products, just because there is not enough empty space between particles.

11.4.10 Pesticide Residues

This subject has been discussed many times within the different trade associations. There is still no firm stance on the matter. I guess that, just as for heavy metals, there has not been a major pressure from consumers and administrations to regulate and issue maximum levels. The most efficient identification methods would be gas or liquid chromatography associated with mass spectrometry.

11.4.11 Mycotoxins

Mycotoxins, especially aflatoxin and ochratoxin, have been of recent concern. Legislations have set maximum levels at 10 ppb in Europe and 20 ppb in the United States. Although aflatoxin has more of a tropical occurrence, ochratoxins have proven to be found in more temperate climates and most countries are in fact affected. They were first discovered in cereals, mainly during long storage in silos, but it is reasonable to see their extension into other plants. Cleanliness seem to be a way to eliminate problems, but their detection still remains a challenge because it is very much dependent on the sampling techniques used.

11.4.12 Other Specific Quality Tests

Piperine levels in pepper, color values (ASTA color value) and capsaicin level in capsicums, and curcumin content in turmeric represent standard determinations in the industry to ensure the quality of supplies. Prices would be based on specific levels, and rugged methods have been developed by the trade associations and prevail worldwide.

11.5 SPICES AND HERBS IN THE FOOD INDUSTRY

In our occidental civilization, and especially in the United States, the food industry has taken over our food habits. It is important, however, to remember that the vast majority

of the planet's population does not follow the same drastic changes and millions if not billions of people still spend time preparing meals, at home, usually following recipes that have been passed down through generations. The world population has many cultures, hence various food histories.

Our relation to food results, in fact, from a synthetic approach among our different senses. First we see a food product, then smell its aroma, long before it enters our mouth; through mastication, its components will finally reach our taste buds. All of our senses generate stimuli that are conveyed to our brain for a final interpretation. At this point we must not forget how important our past experience is. I was about to use the word "training", less noble maybe, but so much closer to reality. Our interpretation of all the visual, olfactive, and taste stimuli relies on years of learning, pleasurable or not. We are used to some products, from the day we were born (and maybe even before that) because of the place we live, the influence of our parents, social pressure, and even religious beliefs. The products we have not been used to would most certainly fall in the dislike category. Each part of the world, sometimes limited to a region, has developed food habits over many centuries. Ironically, they now tend to be referred to as "ethnic food", which in itself carries some kind of exotic connotation, when in fact they could very well be classified as "not what I know", not comparable to what I have been "trained" to like. These aspects need to be remembered when planning any product development. For example, the usual U.S. pickle seasoning, based on dill weed and a touch of cassia (sweetening effect) would not draw much interest in France where the same type of product composition relies on vinegar with tarragon, onion, and pepper for flavor. The opposite situation would certainly bring the same consumer reaction. Humans are prone to follow habits, without realizing they would become more susceptible to boredom. There lies the final threat and history has taught us the high price we may pay for it.

Designing food products involves the active participation of the food chemist, who uses ingredients, just the same way a musician would use notes or the painter colors, to create a combination of sensations to affect as many senses as possible, with the exception of touch and hearing. The goal is to attract the consumer's attention: our attention. Apart from those fortunate enough to enjoy vegetables from the garden, fruits picked just at the right time, meat from animals raised in the open and naturally fed, or fresh seafood from the ocean, the modern-life reality forces the majority of us to feed on products that lack good organoleptic character. The need to supplement that mediocrity with other ingredients is constantly increasing. Luckily enough, Nature helps us in our quest for sensations; spices and herbs bring us what we need, and the spice industry has developed a very diverse line of products to bring flavor to our dishes.

11.5.1 Whole Spices

At the consumer level, spices are sold in small jars, very attractive and also expensive. If you have ever tried to buy saffron (and I mean real), you know what I mean. This activity requires good quality products. As many spices may be sold as such, cleanliness and good visual aspect are a must. It requires extensive sorting capabilities, which will increase the overall cost. Some whole spices such as cinnamon sticks or star anise suffer during transportation. Broken spices may of course be re-worked into ground forms. On the consumer side their usage requires a good knowledge of culinary art and some curiosity, and, above all, time (and inclination) to prepare meals, all becoming rare in today's American society.

11.5.2 Spice Blends

Instead of maintaining stocks of several ingredients, we have seen the development of ready-to-use-blends, in fancy packaging with an alluring name, which we use to sprinkle on all dishes, from meat to soups or vegetables. These blends combine several different spices and herbs for a specific use. It is not a surprise that most contain a sizable amount of pepper, as it has been a world favorite for so long. Blends should also use visual attributes to grab attention, and the freeze-dried herbs, as well as dried vegetable pieces such as bell pepper or carrot, play an essential role. Their contribution to the final taste is limited, but they do bring a nice look. Crushed chilies, with their bright yellow and red flakes, not to mention their pungency, are also a big favorite. The different ingredients may be used in whole, crushed, cracked, diced, or ground forms. The physical aspect would condition both the visual final aspect and their ability to deliver the aromatic constituents that make them valuable. Such blends generally use salt as a carrier (or filler), which allows low pricing. The trend to reduce salt intake has pushed the development of blends without salt. The elaboration of spice blends can easily become an industrial nightmare because of the very wide range of apparent densities in complex blends. Mixing those different ingredients must prevent the breaking up of the most delicate parts (parsley flakes for example), but still ensure a homogeneous mixing and avoid segregation. Overblending is as disastrous as underblending. The packaging steps add more challenges. Transfers and vibrations will rearrange the different ingredients and the final little shaker on the shelf of some convenience store in Nevada may not be quite as the original design intended it to be.

11.5.3 Seasoning Blends

These blends are more complex and include more functional ingredients such as gums, starches, and acids, along with spices, herbs, spices extractives, flavors, and colors. Their main usage is certainly in the meat industry (sausages, patties), but they are also commonly used for snacks, salad dressings, marinades, sauces, and exotic (ethnic) applications. They include spices, along with soluble spice preparations, herbs, and vegetables (onion, garlic), and are added during the final stage of preparation of the food product. The prices of these blends must be low as most industrial users require to save every penny (or fraction of) in their highly competitive market. Duplication of existing blends is the game, as users will switch suppliers when presented with savings. Spices are usually used ground so as to facilitate blending with the more functional ingredients, but cracked pepper or crushed chilies can be of interest for topical applications (an Italian sausage must show pieces of crushed chilies). Some other visual contributors would be herbs flakes for snacks or dips. Spices can also be used in more unconventional associations, like red berries and chili for marinades.

11.6 CONCLUSION

When elaborating a food product, the chemist will naturally turn to ways of making a difference to his composition. In recent years, we have seen a rapid succession of new trends, new health-conscious meals, low-fat, no cholesterol, low sugar, and so on. The new “low-carb” craze is the flavor of the month. How long will it last? Probably until

another high profile "guru" makes his way to the top of the chart. As we remove all the "bad" things from our food, it seems we also take away the fun from it and we are just so lucky that Mother Nature is still around to offer its treasures. Spices and herbs will remain a valuable way to preserve our interest in food.

Today's U.S. food market is also evolving towards constantly more spicy ("hot") food. When walking around the aisles in any grocery store you realize how many spicy products have surfaced in recent years. It almost seems like the intent is to strike the consumer as much as possible, and maybe make him forget the basic taste of the food. Looking back at history, it appears that the Romans tried it some 2000 years ago – I am not so sure this is such a new concept after all. The saturation of the taste induced by high levels of spices, especially pungent varieties, is comparable to looking at a flash, and certainly leads to neuronal pain. Is it still a pleasure? I will leave it to you to decide.

Maybe some of you will decide to embrace a career in the food industry, become a food chemist, create new seasonings or spice blends, carry on the exciting quest for pleasure in food. Why not? You are more than welcome. Working in the spice world is more than a job – it is a passion!

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Section III

Food Sanitation and Establishment Inspection

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

12

FDA GMPs, HACCP, and the Food Code

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12.1 Introduction 224
12.2 Current Good Manufacturing Practice Regulations (CGMPR) 224
12.2.1 Definitions (21 CFR 110.3) 225
12.2.2 Personnel (21 CFR 110.10) 226
12.2.3 Plant and Grounds (21 CFR 110.20) 227
12.2.4 Sanitary Operations (21 CFR 110.35) 228
12.2.5 Sanitary Facilities and Controls (21 CFR 110.37) 230
12.2.6 Equipment and Utensils (21 CFR 110.40) 231
12.2.7 Processes and Controls (21 CFR 110.80) 232
12.2.8 Warehousing and Distribution (21 CFR 110.93) 235
12.2.9 Natural or Unavoidable Defects in Food for Human Use that Present no Health Hazard (21 CFR 110.110) 236
12.3 Hazard Analysis Critical Control Points Regulations (HACCPR) 236
12.3.1 What is HACCP? 236
12.3.2 Need for HACCP 237
12.3.3 Advantages and Plans 237
12.3.4 Hazard Analysis 239
12.3.5 The HACCP Plan 239
12.3.5.1 The Contents of the HACCP Plan 239
12.3.5.2 Signing and Dating the HACCP Plan 240
12.3.6 Sanitation 240
12.3.7 Implementation 240

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

12.4 FDA Food Code 240
12.4.1 Purpose 241
12.4.2 Current Applications of HACCP 242
12.5 Applications 243
References 243

12.1 INTRODUCTION

Nearly 25 years ago, the United States Food and Drug Administration (FDA) started the approach of using umbrella regulations to help the food industries to produce wholesome food as required by the Federal Food, Drug, Cosmetic Act (the Act). In 1986, the FDA promulgated the first umbrella regulations under the title of Good Manufacturing Practice (GMP) regulations (GMPR). Since then, many aspects of the regulations have been revised (FDA).

Traditionally, industry and regulators have depended on spot-checks of manufacturing conditions and random sampling of final products to ensure safe food. The Current Good Manufacturing Practice Regulations (CGMPR) form the basis on which the FDA will inform the food manufacturer about deficiencies in its operations. This approach, however, tends to be reactive, rather than preventive, and can definitely be improved.

For more than 30 years, FDA has been regulating the low acid canned food (LACF) industries with a special set of regulations, many of which are preventive in nature. This action aims at preventing botulism. In the last 30 years, threats from other biological pathogens have increased tremendously. Between 1980 and 1995, FDA has been studying the approach of using Hazard Analysis and Critical Control Points (HACCP). For this approach, FDA uses the LACF regulations as a partial guide. Since 1995, FDA has issued HACCP regulations (HACCPR) (FDA) for the manufacture or production of several types of food products. These include the processing of seafood and fruit/vegetable juices.

Since 1938, when the Act was first passed by Congress, FDA and State regulatory agencies have worked hard to reach a uniform set of codes for the national regulation of food manufacturing industries and state regulation of retail industries associated with food, for example, groceries, restaurants, catering, and so on. In 1993, the first document, titled *Food Code*, was issued jointly by the FDA and state agencies. It has been revised twice since then. This chapter discusses CGMPR, HACCPR, and the *Food Code*.

12.2 CURRENT GOOD MANUFACTURING PRACTICE REGULATIONS (CGMPR)

The Current Good Manufacturing Practice Regulations (CGMPR) cover the topics listed in Table 12.1. These regulations are discussed in detail here. Please note that the word "shall" in a legal document means mandatory and is used routinely in FDA regulations published in the U.S. Code of Federal Regulations (CFR). In this chapter, the words "should" and "must" are used to make for smoother reading. However, this in no way diminishes the legal impact of the original regulations.

TABLE 12.1 Contents of the Current Good Manufacturing Regulations (CGMPR).

21 CFR 110.3	Definitions
21 CFR 110.5	Current good manufacturing practice
21 CFR 110.10	Personnel
21 CFR 110.19	Exclusions
21 CFR 110.20	Plant and grounds
21 CFR 110.35	Sanitary operations
21 CFR 110.37	Sanitary facilities and controls
21 CFR 110.40	Equipment and utensils
21 CFR 110.80	Processes and controls
21 CFR 110.93	Warehousing and distribution

12.2.1 Definitions (21 CFR 110.3)

FDA has provided the following definitions and interpretations for several important terms.

1. *Acid food* or *acidified food* means foods that have an equilibrium pH of 4.6 or below.
2. *Batter* means a semifluid substance, usually composed of flour and other ingredients, into which principal components of food are dipped or with which they are coated, or which may be used directly to form bakery foods.
3. *Blanching*, except for tree nuts and peanuts, means a prepackaging heat treatment of foodstuffs for a sufficient time and at a sufficient temperature to partially or completely inactivate the naturally occurring enzymes and to effect other physical or biochemical changes in the food.
4. *Critical control point* means a point in a food process where there is a high probability that improper control may cause a hazard or filth in the final food or decomposition of the final food.
5. *Food* includes raw materials and ingredients.
6. *Food-contact surfaces* are those surfaces that contact human food and those surfaces from which drainage onto the food or onto surfaces that contact the food ordinarily occurs during the normal course of operations. *Food-contact surfaces* include utensils and food-contact surfaces of equipment.
7. *Lot* means the food produced during a period of time indicated by a specific code.
8. *Microorganisms* means yeasts, molds, bacteria, and viruses and includes, but is not limited to, species having public health significance. The term *undesirable microorganisms* includes those microorganisms that are of public health significance, that promote decomposition of food, or that indicate that food is contaminated with filth.
9. *Pest* refers to any objectionable animals or insects including, but not limited to, birds, rodents, flies, and insect larvae.
10. *Plant* means the building or facility used for the manufacturing, packaging, labeling, or holding of human food.
11. *Quality control operation* means a planned and systematic procedure for taking all actions necessary to prevent food from being adulterated.

12. *Rework* means clean, unadulterated food that has been removed from processing for reasons other than insanitary conditions or that has been successfully reconditioned by reprocessing and that is suitable for use as food.
13. *Safe moisture level* is a level of moisture low enough to prevent the growth of undesirable microorganisms in the finished product under the intended conditions of manufacturing, storage, and distribution. The maximum safe moisture level for a food is based on its water activity, a_w. An a_w will be considered safe for a food if adequate data are available that demonstrate that the food at or below the given a_w will not support the growth of undesirable microorganisms.
14. *Sanitize* means to adequately treat food-contact surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance, and in substantially reducing numbers of other undesirable microorganisms, but without adversely affecting the product or its safety for the consumer.
15. *Water activity* (a_w) is a measure of the free moisture in a food and is the quotient of the water vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature.

12.2.2 Personnel (21 CFR 110.10)

Plant management should take all reasonable measures and precautions to ensure compliance with the following regulations.

1. *Disease Control.* Any person who, by medical examination or supervisory observation, is shown to have an illness, open lesion, including boils, sores, or infected wounds, by which there is a reasonable possibility of food, food-contact surfaces, or food-packaging materials becoming contaminated, should be excluded from any operations which may be expected to result in such contamination until the condition is corrected. Personnel should be instructed to report such health conditions to their supervisors.
2. *Cleanliness.* All persons working in direct contact with food, food-contact surfaces, and food-packaging materials should conform to hygienic practices while on duty. The methods for maintaining cleanliness include, but are not limited to, the following:
   a. Wearing outer garments suitable to the operation to protect against the contamination of food, food-contact surfaces, or food-packaging materials.
   b. Maintaining adequate personal cleanliness.
   c. Washing hands thoroughly (and sanitizing if necessary to protect against contamination with undesirable microorganisms) in an adequate hand-washing facility before starting work, after each absence from the work station, and at any other time when the hands may have become soiled or contaminated.
   d. Removing all unsecured jewelry and other objects that might fall into food, equipment, or containers, and removing hand jewelry that cannot be adequately sanitized during periods in which food is manipulated by hand. If such hand jewelry cannot be removed, it may be covered by material which can be maintained in an intact, clean, and sanitary condition and which effectively protects against their contamination of the food, food-contact surfaces, or food-packaging materials.
   e. Maintaining gloves, if they are used in food handling, in an intact, clean, and sanitary condition. The gloves should be of an impermeable material.

f. Wearing, where appropriate, hairnets, headbands, caps, beard covers, or other effective hair restraints.
g. Storing clothing or other personal belongings in areas other than where food is exposed or where equipment or utensils are washed.
h. Confining the following personal practices to areas other than where food may be exposed or where equipment or utensils are washed: eating food, chewing gum, drinking beverages, or using tobacco.
i. Taking any other necessary precautions to protect against contamination of food, food-contact surfaces, or food-packaging materials with microorganisms or foreign substances including, but not limited to, perspiration, hair, cosmetics, tobacco, chemicals, and medicines applied to the skin.

3. *Education and Training*. Personnel responsible for identifying sanitation failures or food contamination should have a background of education or experience, to provide a level of competency necessary for production of clean and safe food. Food handlers and supervisors should receive appropriate training in proper food handling techniques and food-protection principles and should be informed of the danger of poor personal hygiene and insanitary practices.
4. *Supervision*. Responsibility for assuring compliance by all personnel with all legal requirements should be clearly assigned to competent supervisory personnel.

12.2.3 Plant and Grounds (21 CFR 110.20)

1. *Grounds*. The grounds surrounding a food plant that are under the control of the plant manager should be kept in a condition that will protect against the contamination of food. The methods for adequate maintenance of grounds include, but are not limited to, the following.
   a. Properly storing equipment, removing litter and waste, and cutting weeds or grass within the immediate vicinity of the plant buildings or structures that may constitute an attractant, breeding place or harborage for pests.
   b. Maintaining roads, yards, and parking lots so that they do not constitute a source of contamination in areas where food is exposed.
   c. Adequately draining areas that may contribute contamination to food by seepage or foot-borne filth, or by providing a breeding place for pests.
   d. Operating systems for waste treatment and disposal in an adequate manner so that they do not constitute a source of contamination in areas where food is exposed. If the plant grounds are bordered by grounds not under the operator's control and not maintained in an acceptable manner, steps must be taken to exclude pests, dirt, and filth that may be a source of food contamination. Implement inspection, extermination, or other countermeasures.
2. *Plant Construction and Design*. Plant buildings and structures should be suitable in size, construction, and design to facilitate maintenance and sanitary operations for food-manufacturing purposes. The plant and facilities should:
   a. Provide sufficient space for such placement of equipment and storage of materials as is necessary for the maintenance of sanitary operations and the production of safe food.

b. Take proper precautions to reduce the potential for contamination of food, food-contact surfaces, or food-packaging materials with microorganisms, chemicals, filth, or other extraneous material. The potential for contamination may be reduced by adequate food safety controls and operating practices or effective design, including the separation of operations in which contamination is likely to occur, by one or more of the following means: location, time, partition, air flow, enclosed systems, or other effective means.
c. Take proper precautions to protect food in outdoor bulk fermentation vessels by any effective means, including:
   – Using protective coverings,
   – Controlling areas over and around the vessels to eliminate harborages for pests,
   – Checking on a regular basis for pests and pest infestation,
   – Skimming the fermentation vessels, as necessary,
d. Be constructed in such a manner that floors, walls, and ceilings may be adequately cleaned and kept clean and kept in good repair; that drip or condensate from fixtures, ducts and pipes does not contaminate food, food-contact surfaces, or food-packaging materials; and that aisles or working spaces are provided between equipment and walls and are adequately unobstructed and of adequate width to permit employees to perform their duties and to protect against contaminating food or food-contact surfaces with clothing or personal contact.
e. Provide adequate lighting in hand-washing areas, dressing and locker rooms, and toilet rooms and in all areas where food is examined, processed, or stored and where equipment or utensils are cleaned; and provide safety-type light bulbs, fixtures, skylights, or other glass suspended over exposed food in any step of preparation or otherwise protect against food contamination in case of glass breakage.
f. Provide adequate ventilation or control equipment to minimize odors and vapors (including steam and noxious fumes) in areas where they may contaminate food; and locate and operate fans and other air-blowing equipment in a manner that minimizes the potential for contaminating food, food-packaging materials, and food-contact surfaces.
g. Provide, where necessary, adequate screening or other protection against pests.

12.2.4 Sanitary Operations (21 CFR 110.35)

1. *General Maintenance*. Buildings, fixtures, and other physical facilities of the plant should be maintained in a sanitary condition and should be kept in repair sufficient to prevent food from becoming adulterated within the meaning of the Act. Cleaning and sanitizing of utensils and equipment should be conducted in a manner that protects against contamination of food, food-contact surfaces, or food-packaging materials.
2. *Substances Used in Cleaning and Sanitizing; Storage of Toxic Materials*.
   a. Cleaning compounds and sanitizing agents used in cleaning and sanitizing procedures should be free from undesirable microorganisms and should be safe and adequate under the conditions of use. Compliance with this requirement may be

verified by any effective means including purchase of these substances under a supplier's guarantee or certification, or examination of these substances for contamination. Only the following toxic materials may be used or stored in a plant where food is processed or exposed:

- Those required to maintain clean and sanitary conditions;
- Those necessary for use in laboratory testing procedures;
- Those necessary for plant and equipment maintenance and operation; and
- Those necessary for use in the plant's operations.

b. Toxic cleaning compounds, sanitizing agents, and pesticide chemicals should be identified, held, and stored in a manner that protects against contamination of food, food-contact surfaces, or food-packaging materials.

3. *Pest Control.* No pests should be allowed in any area of a food plant. Guard or guide dogs may be allowed in some areas of a plant if the presence of the dogs is unlikely to result in contamination of food, food-contact surfaces, or food-packaging materials. Effective measures should be taken to exclude pests from the processing areas and to protect against the contamination of food on the premises by pests. The use of insecticides or rodenticides is permitted only under precautions and restrictions that will protect against the contamination of food, food-contact surfaces, and food-packaging materials.
4. *Sanitation of Food-Contact Surfaces.* All food-contact surfaces, including utensils and food-contact surfaces of equipment, should be cleaned as frequently as necessary to protect against contamination of food.
   a. Food-contact surfaces used for manufacturing or holding low-moisture food should be in a dry, sanitary condition at the time of use. When the surfaces are wet-cleaned, they should, when necessary, be sanitized and thoroughly dried before subsequent use.
   b. In wet processing, when cleaning is necessary to protect against the introduction of microorganisms into food, all food-contact surfaces should be cleaned and sanitized before use and after any interruption during which the food-contact surfaces may have become contaminated. Where equipment and utensils are used in a continuous production operation, the utensils and food-contact surfaces of the equipment should be cleaned and sanitized as necessary.
   c. Non-food-contact surfaces of equipment used in the operation of food plants should be cleaned as frequently as necessary to protect against contamination of food.
   d. Single-service articles (such as utensils intended for one-time use, paper cups, and paper towels) should be stored in appropriate containers and should be handled, dispensed, used, and disposed of in a manner that protects against contamination of food or food-contact surfaces.
   e. Sanitizing agents should be adequate and safe under conditions of use. Any facility, procedure, or machine is acceptable for cleaning and sanitizing equipment and utensils if it is established that the facility, procedure, or machine will routinely render equipment and utensils clean and provide adequate cleaning and sanitizing treatment.

5. *Storage and Handling of Cleaned Portable Equipment and Utensils*. Cleaned and sanitized portable equipment with food-contact surfaces and utensils should be stored in a location and manner that protects food-contact surfaces from contamination.

12.2.5 Sanitary Facilities and Controls (21 CFR 110.37)

Each plant should be equipped with adequate sanitary facilities and accommodations including, but not limited to the following.

1. *Water Supply*. The water supply should be sufficient for the operations intended and should be derived from an adequate source. Any water that contacts food or food-contact surfaces should be safe and of adequate sanitary quality. Running water at a suitable temperature, and under pressure as needed, should be provided in all areas where required for the processing of food, for the cleaning of equipment, utensils, and food-packaging materials, or for employee sanitary facilities.
2. *Plumbing*. Plumbing should be of adequate size and design and adequately installed and maintained to
   a. Carry sufficient quantities of water to required locations throughout the plant;
   b. Properly convey sewage and liquid disposable waste from the plant;
   c. Avoid constituting a source of contamination to food, water supplies, equipment, or utensils or creating an unsanitary condition;
   d. Provide adequate floor drainage in all areas where floors are subject to flooding-type cleaning or where normal operations release or discharge water or other liquid waste on the floor; and
   e. Provide that there is no backflow from, or cross-connection between, piping systems that discharge wastewater or sewage and piping systems that carry water for food or food manufacturing.
3. *Sewage Disposal*. Sewage disposal should be made into an adequate sewerage system or disposed of through other adequate means.
4. *Toilet Facilities*. Each plant should provide its employees with adequate, readily accessible toilet facilities. Compliance with this requirement may be accomplished by
   a. Maintaining the facilities in a sanitary condition;
   b. Keeping the facilities in good repair at all times;
   c. Providing self-closing doors;
   d. Providing doors that do not open into areas where food is exposed to airborne contamination, except where alternate means have been taken to protect against such contamination (such as double doors or positive air-flow systems).
5. *Hand-Washing Facilities*. Hand-washing facilities should be adequate and convenient and be furnished with running water at a suitable temperature. Compliance with this requirement may be accomplished by providing the following.
   a. Hand-washing and, where appropriate, hand-sanitizing facilities at each location in the plant where good sanitary practices require employees to wash and/or sanitize their hands.
   b. Effective hand-cleaning and sanitizing preparations.

c. Sanitary towel service or suitable drying devices.
d. Devices or fixtures, such as water control valves, so designed and constructed to protect against recontamination of clean, sanitized hands.
e. Readily understandable signs directing employees handling unprotected food, unprotected food-packaging materials, of food-contact surfaces to wash and, where appropriate, sanitize their hands before they start work, after each absence from post of duty, and when their hands may have become soiled or contaminated. These signs may be posted in the processing room(s) and in all other areas where employees may handle such food, materials, or surfaces.
f. Refuse receptacles that are constructed and maintained in a manner that protects against contamination of food.

6. *Rubbish and Offal Disposal*. Rubbish and any offal should be so conveyed, stored, and disposed of as to minimize the development of odor, minimize the potential for the waste becoming an attractant and harborage or breeding place for pests, and protect against contamination of food, food-contact surfaces, water supplies, and ground surfaces.

12.2.6 Equipment and Utensils (21 CFR 110.40)

1. All plant equipment and utensils should be so designed and of such material and workmanship as to be adequately cleanable, and should be properly maintained. The design, construction, and use of equipment and utensils should preclude the adulteration of food with lubricants, fuel, metal fragments, contaminated water, or any other contaminants. All equipment should be so installed and maintained as to facilitate the cleaning of the equipment and of all adjacent spaces. Food-contact surfaces should be corrosion-resistant when in contact with food. They should be made of nontoxic materials and designed to withstand the environment of their intended use and the action of food, and, if applicable, cleaning compounds and sanitizing agents. Food-contact surfaces should be maintained to protect food from being contaminated by any source, including unlawful indirect food additives.
2. Seams on food-contact surfaces should be smoothly bonded or maintained so as to minimize accumulation of food particles, dirt, and organic matter and thus minimize the opportunity for growth of microorganisms.
3. Equipment that is in the manufacturing or food-handling area and that does not come into contact with food should be so constructed that it can be kept in a clean condition.
4. Holding, conveying, and manufacturing systems, including gravimetric, pneumatic, closed, and automated systems, should be of a design and construction that enables them to be maintained in an appropriate sanitary condition.
5. Each freezer and cold storage compartment used to store and hold food capable of supporting growth of microorganisms should be fitted with an indicating thermometer, temperature-measuring device, or temperature-recording device so installed as to show the temperature accurately within the compartment, and should be fitted with an automatic control for regulating temperature or with an automatic alarm system to indicate a significant temperature change in a manual operation.

6. Instruments and controls used for measuring, regulating, or recording temperatures, pH, acidity, water activity, or other conditions that control or prevent the growth of undesirable microorganisms in food should be accurate and adequately maintained, and adequate in number for their designated uses.
7. Compressed air or other gases mechanically introduced into food or used to clean food-contact surfaces or equipment should be treated in such a way that food is not contaminated with unlawful indirect food additives.

12.2.7 Processes and Controls (21 CFR 110.80)

All operations in the receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, and storing of food should be conducted in accordance with adequate sanitation principles. Appropriate quality control operations should be employed to ensure that food is suitable for human consumption and that food-packaging materials are safe and suitable. Overall sanitation of the plant should be under the supervision of one or more competent individuals assigned responsibility for this function. All reasonable precautions should be taken to ensure that production procedures do not contribute contamination from any source. Chemical, microbial, or extraneous material testing procedures should be used where necessary to identify sanitation failures or possible food contamination. All food that has become contaminated to the extent that it is adulterated within the meaning of the Act should be rejected, or if permissible, treated or processed to eliminate the contamination.

1. *Raw Materials and Other Ingredients.*
   a. Raw materials and other ingredients should be inspected and segregated or otherwise handled as necessary to ascertain that they are clean and suitable for processing into food and should be stored under conditions that will protect against contamination and minimize deterioration. Raw materials should be washed or cleaned as necessary to remove soil or other contamination. Water used for washing, rinsing, or conveying food should be safe and of adequate sanitary quality. Water may be reused for washing, rinsing, or conveying food if it does not increase the level of contamination of the food. Containers and carriers of raw materials should be inspected on receipt to ensure that their condition has not contributed to the contamination or deterioration of food.
   b. Raw materials and other ingredients should either not contain levels of microorganisms that may produce food poisoning or other disease in humans, or they should be pasteurized or otherwise treated during manufacturing operations so that they no longer contain levels that would cause the product to be adulterated within the meaning of the act. Compliance with this requirement may be verified by any effective means, including purchasing raw materials and other ingredients under a supplier's guarantee or certification.
   c. Raw materials and other ingredients susceptible to contamination with aflatoxin or other natural toxins should comply with current FDA regulations, guidelines, and action levels for poisonous or deleterious substances before these materials or ingredients are incorporated into finished food. Compliance with this requirement may be accomplished by purchasing raw materials and other ingredients

under a supplier's guarantee or certification, or may be verified by analyzing these materials and ingredients for aflatoxins and other natural toxins.

d. Raw materials, other ingredients, and rework susceptible to contamination with pests, undesirable microorganisms, or extraneous material should comply with applicable FDA regulations, guidelines, and defect action levels for natural or unavoidable defects if a manufacturer wishes to use the materials in manufacturing food. Compliance with this requirement may be verified by any effective means, including purchasing the materials under a supplier's guarantee or certification, or examination of these materials for contamination.

e. Raw materials, other ingredients, and rework should be held in bulk, or in containers designed and constructed so as to protect against contamination and should be held at such temperature and relative humidity as to prevent the food from becoming adulterated. Material scheduled for rework should be identified as such.

f. Frozen raw materials and other ingredients should be kept frozen. If thawing is required prior to use, it should be done in a manner that prevents the raw materials and other ingredients from becoming adulterated.

g. Liquid or dry raw materials and other ingredients received and stored in bulk form should be held in a manner that protects against contamination.

2. *Manufacturing operations.*

a. Equipment and utensils and finished food containers should be maintained in an acceptable condition through appropriate cleaning and sanitizing, as necessary. Insofar as necessary, equipment should be taken apart for thorough cleaning.

b. All food manufacturing, including packaging and storage, should be conducted under such conditions and controls as are necessary to minimize the potential for the growth of microorganisms, or for the contamination of food. One way to comply with this requirement is careful monitoring of physical factors such as time, temperature, humidity, a_w, pH, pressure, flow rate, and manufacturing operations such as freezing, dehydration, heat processing, acidification, and refrigeration to ensure that mechanical breakdowns, time delays, temperature fluctuations, and other factors do not contribute to the decomposition or contamination of food.

c. Food that can support the rapid growth of undesirable microorganisms, particularly those of public health significance, should be held in a manner that prevents the food from becoming contaminated. Compliance with this requirement may be accomplished by any effective means, including:

   – Maintaining refrigerated foods at 41°F (5°C) or below as appropriate for the particular food involved;
   – Maintaining frozen foods in a frozen state;
   – Maintaining hot foods at 140°F (60°C) or above;
   – Heat treating acid or acidified foods to destroy mesophilic microorganisms when those foods are to be held in hermetically sealed containers at ambient temperatures.

d. Measures such as sterilizing, irradiating, pasteurizing, freezing, refrigerating, controlling pH, or controlling a_w that are taken to destroy or prevent the growth of undesirable microorganisms, particularly those of public health

significance, should be adequate under the conditions of manufacture, handling, and distribution to prevent food from being adulterated.

e. Work-in-process should be handled in a manner that protects against contamination.

f. Effective measures should be taken to protect finished food from contamination by raw materials, other ingredients, or refuse. When raw materials, other ingredients, or refuse are unprotected, they should not be handled simultaneously in a receiving, loading, or shipping area if that handling could result in contaminated food. Food transported by conveyor should be protected against contamination as necessary.

g. Equipment, containers, and utensils used to convey, hold, or store raw materials, work-in-process, rework, or food should be constructed, handled, and maintained during manufacturing or storage in a manner that protects against contamination.

h. Effective measures should be taken to protect against the inclusion of metal or other extraneous material in food. Compliance with this requirement may be accomplished by using sieves, traps, magnets, electronic metal detectors, or other suitable effective means.

i. Food, raw materials, and other ingredients that are adulterated should be disposed of in a manner that protects against the contamination of other food. If the adulterated food is capable of being reconditioned, it should be reconditioned using a method that has been proven to be effective or it should be reexamined and found not to be adulterated before being incorporated into other food.

j. Mechanical manufacturing steps such as washing, peeling, trimming, cutting, sorting and inspecting, mashing, dewatering, cooling, shredding, extruding, drying, whipping, defatting, and forming should be performed so as to protect food against contamination. Compliance with this requirement may be accomplished by providing adequate physical protection of food from contaminants that may drip, drain, or be drawn into the food. Protection may be provided by adequate cleaning and sanitizing of all food-contact surfaces, and by using time and temperature controls at and between each manufacturing step.

k. Heat blanching, when required in the preparation of food, should be effected by heating the food to the required temperature, holding it at this temperature for the required time, and then either rapidly cooling the food or passing it to subsequent manufacturing without delay. Thermophilic growth and contamination in blanchers should be minimized by the use of adequate operating temperatures and by periodic cleaning. Where the blanched food is washed prior to filling, water used should be safe and of adequate sanitary quality.

l. Batters, breading, sauces, gravies, dressings, and other similar preparations should be treated or maintained in such a manner that they are protected against contamination. Compliance with this requirement may be accomplished by any effective means, including one or more of the following:
   - Using ingredients free of contamination;
   - Employing adequate heat processes where applicable;
   - Using adequate time and temperature controls;
   - Providing adequate physical protection of components from contaminants that may drip, drain, or be drawn into them;

- Cooling to an adequate temperature during manufacturing;
- Disposing of batters at appropriate intervals to protect against the growth of microorganisms.

m. Filling, assembling, packaging, and other operations should be performed in such a way that the food is protected against contamination. Compliance with this requirement may be accomplished by any effective means, including:
   - Use of a quality control operation in which the critical control points are identified and controlled during manufacturing;
   - Adequate cleaning and sanitizing of all food-contact surfaces and food containers;
   - Using materials for food containers and food-packaging materials that are safe and suitable;
   - Providing physical protection from contamination, particularly airborne contamination;
   - Using sanitary handling procedures.

n. Food such as, but not limited to, dry mixes, nuts, intermediate-moisture food, and dehydrated food, that relies on the control of a_w for preventing the growth of undesirable microorganisms should be processed to and maintained at a safe moisture level. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
   - Monitoring the a_w of food;
   - Controlling the soluble solids/water ratio in finished food;
   - Protecting finished food from moisture pickup, by use of a moisture barrier or by other means, so that the a_w of the food does not increase to an unsafe level.

o. Food, such as, but not limited to, acid and acidified food, which relies principally on the control of pH for preventing the growth of undesirable microorganisms should be monitored and maintained at a pH of 4.6 or below. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
   - Monitoring the pH of raw materials, food-in-process, and finished food;
   - Controlling the amount of acid or acidified food added to low-acid food.

p. When ice is used in contact with food, it should be made from water that is safe and of adequate sanitary quality, and should be used only if it has been manufactured in accordance with current good manufacturing practice.

q. Food-manufacturing areas and equipment used for manufacturing human food should not be used to manufacture nonhuman-food-grade animal feed or inedible products, unless there is no reasonable possibility for the contamination of the human food.

12.2.8 Warehousing and Distribution (21 CFR 110.93)

Storage and transportation of finished food should be under conditions that will protect food against physical, chemical, and microbial contamination as well as against deterioration of the food and the container.

12.2.9 Natural or Unavoidable Defects in Food for Human Use that Present no Health Hazard (21 CFR 110.110)

1. Some foods, even when produced under current good manufacturing practice, contain natural or unavoidable defects that at low levels are not hazardous to health. FDA establishes maximum levels for these defects in foods produced under current good manufacturing practice and uses these levels in deciding whether to recommend regulatory action.
2. Defect action levels are established for foods whenever it is necessary and feasible to do so. These levels are subject to change upon the development of new technology or the availability of new information.
3. The mixing of a food containing defects above the current defect action level with another lot of food is not permitted and renders the final food adulterated within the meaning of the Act, regardless of the defect level of the final food.
4. A compilation of the current defect action levels for natural or unavoidable defects in food for human use that present no health hazard may be obtained from the FDA for printed or electronic versions.

12.3 HAZARD ANALYSIS CRITICAL CONTROL POINTS REGULATIONS (HACCPR)

In 1997, FDA adopted a food safety program that was developed nearly 30 years ago for astronauts, and is now applying it to seafood, and fruit and vegetable juices. The agency intends to eventually use it for much of the U.S. food supply. The program for astronauts focuses on preventing hazards that could cause foodborne illnesses by applying science-based controls, from raw material to finished products. FDA's new system will do the same.

Many principles of this new system, now called Hazard Analysis and Critical Control Points (HACCP), are already in place in the FDA-regulated low-acid canned food industry. Since 1997, FDA has mandated HACCP for the processing of seafood, fruit juices, and vegetable juices. Also, FDA has incorporated HACCP into its *Food Code*, a document that gives guidance to and serves as model legislation for state and territorial agencies that license and inspect food service establishments, retail food stores, and food vending operations in the United States.

FDA is now considering developing regulations that would establish HACCP as the food safety standard throughout other areas of the food industry, including both domestic and imported food products. HACCP has been endorsed by the National Academy of Sciences, the Codex Alimentarius Commission (an international, standard-setting organization), and the National Advisory Committee on Microbiological Criteria for Foods. Several U.S. food companies already use the system in their manufacturing processes, and it is in use in other countries, including Canada.

12.3.1 What is HACCP?

HACCP involves seven principles.

1. *Analyze Hazards*. Potential hazards associated with a food and measures to control those hazards are identified. The hazard could be biological, such as a

microbe; chemical, such as a toxin; or physical, such as ground glass or metal fragments.

2. *Identify Critical Control Points*. These are points in a food's production – from its raw state through processing and shipping to consumption by the consumer – at which the potential hazard can be controlled or eliminated. Examples are cooking, cooling, packaging, and metal detection.
3. *Establish Preventive Measures with Critical Limits for Each Control Point*. For a cooked food, for example, this might include setting the minimum cooking temperature and time required to ensure the elimination of any harmful microbes.
4. *Establish Procedures to Monitor the Critical Control Points*. Such procedures might include determining how and by whom cooking time and temperature should be monitored.
5. *Establish Corrective Actions* to be taken when monitoring shows that a critical limit has not been met – for example, reprocessing or disposing of food if the minimum cooking temperature is not met.
6. *Establish Procedures to Verify that the System is Working Properly* – for example, testing time-and-temperature recording devices to verify that a cooking unit is working properly.
7. *Establish Effective Record Keeping* to document the HACCP system. This would include records of hazards and their control methods, the monitoring of safety requirements and action taken to correct potential problems.

Each of these principles must be backed by sound scientific knowledge such as published microbiological studies on time and temperature factors for controlling foodborne pathogens.

12.3.2 Need for HACCP

New challenges to the U.S. food supply have prompted FDA to consider adopting a HACCP-based food safety system on a wider basis. One of the most important challenges is the increasing number of new food pathogens. For example, between 1973 and 1988, bacteria not previously recognized as important causes of foodborne illness such as *Escherichia coli* O157:H7 and *Salmonella enteritidis* became more widespread. There is also increasing public health concerns about chemical contamination of food: for example, the effects of lead in food on the nervous system.

Another important factor is the fact that the size of the food industry and the diversity of products and processes have grown tremendously – in the amount of domestic food manufactured and the number and kinds of foods imported. At the same time, FDA and state and local agencies have the same limited level of resources to ensure food safety. The need for HACCP in the United States, particularly in the seafood industry, is further fueled by the growing trend in international trade for worldwide equivalence of food products and the Codex Alimentarius Commission's adoption of HACCP as the international standard for food safety.

12.3.3 Advantages and Plans

HACCP offers a number of advantages over previous systems. Most importantly, HACCP

1. Focuses on identifying and preventing hazards from contaminating food;
2. Is based on sound science;

3. Permits more efficient and effective government oversight, primarily because the record keeping allows investigators to see how well a firm is complying with food safety laws over a period rather than how well it is doing on any given day;
4. Places responsibility for ensuring food safety appropriately on the food manufacturer or distributor;
5. Helps food companies compete more effectively in the world market; and
6. Reduces barriers to international trade.

The following are the seven steps used in HACCP plan development.

1. Preliminary steps:
   a. General information;
   b. Describe the food;
   c. Describe the method of distribution and storage;
   d. Identify the intended use and consumer;
   e. Develop a flow diagram.
2. Hazard Analysis Worksheet:
   a. Set up the Hazard Analysis Worksheet;
   b. Identify the potential species-related hazards;
   c. Identify the potential process-related hazards;
   d. Complete the Hazard Analysis Worksheet;
   e. Understand the potential hazard;
   f. Determine if the potential hazard is significant;
   g. Identify the critical control points (CCP).
3. HACCP Plan Form:
   a. Complete the HACCP Plan Form;
   b. Set the critical limits (CL).
4. Establish monitoring procedures:
   a. What;
   b. How;
   c. Frequency;
   d. Who.
5. Establish corrective action procedures.
6. Establish a record keeping system.
7. Establish verification procedures.

It is important to remember that, apart from the HACCPR promulgated for seafood and juices, the implementation of HACCP by other categories of food processing is voluntary. However, the FDA and various types of food processors are working together so that eventually HACCPR will become available for many other food processing systems under FDA jurisdiction. Using the HACCPR for seafood processing as a guide, the following

discussion for a HACCP plan applies to all categories of food products being processed in United States.

12.3.4 Hazard Analysis

Every processor should conduct a hazard analysis to determine whether there are food safety hazards that are reasonably likely to occur for each kind of product processed by that processor and to identify the preventive measures that the processor can apply to control those hazards. Such food safety hazards can be introduced both within and outside the processing plant environment, including food safety hazards that can occur before, during, and after harvest. A food safety hazard that is reasonably likely to occur is one for which a prudent processor would establish controls because experience, illness data, scientific reports, or other information provide a basis to conclude that there is a reasonable possibility that it will occur in the particular type of product being processed in the absence of those controls.

12.3.5 The HACCP Plan

Every processor should have and implement a written HACCP plan whenever a hazard analysis reveals one or more food safety hazards that are reasonably likely to occur. A HACCP plan should be specific to:

1. Each location where products are processed by that processor; and
2. Each kind of product processed by the processor.

The plan may group kinds of products together, or group kinds of production methods together, if the food safety hazards, critical control points, critical limits, and procedures required to be identified and performed are identical for all products so grouped or for all production methods so grouped.

12.3.5.1 The Contents of the HACCP Plan. The HACCP plan should, at a minimum do the following.

1. List the food safety hazards that are reasonably likely to occur, as identified, and that thus must be controlled for each product. Consideration should be given to whether any food safety hazards are reasonably likely to occur as a result of the following: natural toxins; microbiological contamination; chemical contamination; pesticides; drug residues; decomposition in products where a food safety hazard has been associated with decomposition; parasites, where the processor has knowledge that the parasite-containing product will be consumed without a process sufficient to kill the parasites; unapproved use of direct or indirect food or color additives; and physical hazards.
2. List the critical control points for each of the identified food safety hazards, including as appropriate: critical control points designed to control food safety hazards that could be introduced in the processing plant environment; and critical control points designed to control food safety hazards introduced outside the processing plant environment, including food safety hazards that occur before, during, and after harvest.

3. List the critical limits that must be met at each of the critical control points.
4. List the procedures, and frequency thereof, that will be used to monitor each of the critical control points to ensure compliance with the critical limits.
5. Include any corrective action plans that have been developed to be followed in response to deviations from critical limits at critical control points.
6. List the verification procedures, and frequency thereof, that the processor will use.
7. Provide for a record keeping system that documents the monitoring of the critical control points. The records should contain the actual values and observations obtained during monitoring.

12.3.5.2 Signing and Dating the HACCP Plan. The HACCP plan should be signed and dated

- Either by the most responsible individual on site at the processing facility or by a higher-level official of the processor – this signature should signify that the HACCP plan has been accepted for implementation by the firm;
- Upon initial acceptance; upon any modification; and upon verification of the plan.

12.3.6 Sanitation

Sanitation controls (FDA) may be included in the HACCP plan. However, to the extent that they are otherwise monitored, they need not be included in the HACCP plan.

12.3.7 Implementation

This book is not the proper forum to discuss in detail the implementation of HACCPR. Readers interested in additional information on HACCP should visit the FDA HACCP website at http://vm.cfsan.fda.gov/, which lists all the currently available documents on the subject.

12.4 FDA FOOD CODE

The FDA *Food Code* (the Code) (FDA) is an essential reference that guides retail outlets such as restaurants and grocery stores and institutions such as nursing homes on how to prevent foodborne illness. Local, state, and federal regulators use the FDA *Food Code* as a model to help develop or update their own food safety rules and to be consistent with national food regulatory policy. Also, many of the over one million retail food establishments apply *Food Code* provisions to their own operations. The *Food Code* is updated every two years, to coincide with the biennial meeting of the Conference for Food Protection. The conference is a group of representatives from regulatory agencies at all levels of government, the food industry, academia, and consumer organizations that works to improve food safety at the retail level (FDA). A brief discussion of the Code is provided below. Further information may be obtained from the FDA.

The Code establishes definitions; sets standards for management and personnel, food operations, and equipment and facilities; and provides for food establishment plan review, permit issuance, inspection, employee restriction, and permit suspension. The

Code discusses the good manufacturing practices for equipment, utensils, linens, water, plumbing, waste, physical facilities, poisonous or toxic materials, compliance, and enforcement. The Code also provides guidelines on food establishment inspection, HACCP guidelines, food processing criteria, model forms, guides, and other aids.

Although this guide is designed for retail food protection, more than half of the data included are directly applicable to food processing plants, for example, equipment design (cleanability), CIP system, detergents and sanitizers, refrigeration and freezing storage parameters, water requirements, precautions against "backflow" (air, valve, etc.), personnel health and hygiene, rest rooms and accessories, pest control, storage of toxic chemicals, inspection forms, inspection procedures, and many more.

The Code consists of eight chapters and seven annexes. The annex that covers inspection of a food establishment applies equally as well to both retail food protection and to sanitation in food processing. According to the Code, the components of an inspection would usually include the following elements:

- Introduction;
- Program planning;
- Staff training;
- Conducting the inspection;
- Inspection documentation;
- Inspection report;
- Administrative procedures by the state/local authorities;
- Temperature-measuring devices;
- Calibration procedures;
- HACCP inspection data form;
- Food establishment inspection report;
- FDA electronic inspection system;
- Establishment scoring.

Details of these items will not be discussed here; some are further explored in various chapters in this book (please consult the index for specific topics). Instead, the next two sections trace the history and practices of food establishment inspection and how basic sanitation controls are slowly evolving into the prerequisites for HACCP plans in both retail food protection *and* food processing plants.

12.4.1 Purpose

A principal goal to be achieved by a food establishment inspection is to prevent foodborne disease. Inspection is the primary tool a regulatory agency has for detecting procedures and practices that may be hazardous and for taking actions to correct deficiencies. Laws and ordinances based on the *Food Code* provide inspectors with science-based rules for food safety. The *Food Code* provides regulatory agencies with guidance on planning, scheduling, conducting, and evaluating inspections. It supports programs by providing recommendations for training and equipping the inspection staff, and attempts to enhance the effectiveness of inspections by stressing the importance of communication

and information exchange during regulatory visits. Inspections aid the food-service industry in the following ways.

1. Serving as educational sessions on specific Code requirements as they apply to an establishment and its operation;
2. Conveying new food safety information to establishment management and providing an opportunity for management to ask questions about general food safety matters; and
3. Providing a written report to the establishment's permit holder or person in charge so that the responsible person can bring the establishment into conformance with the Code.

12.4.2 Current Applications of HACCP

Inspections have been a part of food safety regulatory activities since the earliest days of public health. Traditionally, inspections have focused primarily on sanitation. Each inspection is unique in terms of the establishment's management, personnel, menu, recipes, operations, size, population served, and many other considerations.

Changes to the traditional inspection process were first suggested in the 1970s. The terms "traditional" or "routine" inspection have been used to describe periodic inspections conducted as part of an ongoing regulatory scheme. A full range of approaches was tried and many were successful in managing a transition to a new inspection philosophy and format. During the 1980s, many progressive jurisdictions started employing the HACCP approach to refocus their inspections. The term "HACCP approach" inspection is used to describe an inspection using the "Hazard Analysis and Critical Control Point" concept. Food safety is the primary focus of a HACCP approach inspection. One lesson learned was that good communication skills on the part of the person conducting an inspection are essential.

FDA has taught thousands of state and local inspectors the principles and applications of HACCP since the 1980s. The State Training Branch and the FDA Regional Food Specialists have provided two-day to week-long courses on the scientific principles on which HACCP is based, the practical application of these principles including field exercises, and reviews of case studies. State and local jurisdictions have also offered many training opportunities for HACCP.

A recent review of state and local retail food protection agencies shows that HACCP is being applied in the following ways.

1. *Formal Studies* – An inspector is trained in HACCP and is using the concepts to study food hazards in establishments. These studies actually follow foods from delivery to service and involve the write-up of data obtained (flow charts, cooling curves, etc.).
2. *Routine Use* – State has personnel trained in HACCP and is using the hazard analysis concepts to more effectively discover hazards during routine inspections.
3. *Consultation* – HACCP-trained personnel are consulting with industry and assisting them in designing and implementing internal HACCP systems and plans.
4. *Alternative Use* – Jurisdiction has used HACCP to change inspection forms or regulations.

5. *Risk-Based* – Jurisdiction has prioritized the inventory of establishments and set inspection frequency using a hazard assessment.
6. *Training* – Jurisdiction is in the active process of training inspectors in the HACCP concepts.

Personnel of every sort of food establishment should have one or several copies of the *Food Code* readily available for frequent consultation.

12.5 APPLICATIONS

The sanitary requirements in the CGMPR and the *Food Code* serve as the framework for the chapters in this book. The HACCPR will be touched on when they help to clarify the discussion. Essentially, this book shows how to implement the umbrella regulations provided under the CGMPR. Each chapter handles one aspect of these complicated regulations. Most chapters discuss the regulations applicable to all types of food products being processed. Several chapters concentrate on the sanitary requirements from the perspectives of the processing of a specific category of food.

REFERENCES

FDA Official Website http://www.fda.gov/.

Current Good Manufacturing Practice in Manufacturing, Packing, or Holding Human Food. 21 CFR 110.

Hazard Analysis and Critical Control Point (HACCP) Systems. 21 CFR 120.

Food Code www.cfsan.fda.gov/~dms/foodcode.html.

13

Food Establishment Inspection

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13.1 Introduction 246
13.1.1 Preparation 246
13.1.2 Food Registration 246
13.2 Personnel 246
13.2.1 Employees 247
13.3 Plants and Grounds 247
13.3.1 Environment 247
13.3.2 Plant Construction, Design, and Maintenance 248
13.3.3 Waste Disposal 248
13.3.4 Plant Services 248
13.4 Raw Materials 249
13.5 Equipment and Utensils 249
13.6 Manufacturing Process 251
13.6.1 Ingredient Handling 251
13.6.2 Formulas 251
13.6.3 Food Additives 252
13.6.4 Color Additives 253
13.6.5 Quality Control 253
13.6.5.1 Inspection System 254
13.6.5.2 Laboratory Tests 254

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

13.6.5.3 Manufacturing Code System 254
13.6.6 Packaging and Labeling 254
13.6.6.1 Quantity of Contents 255
13.6.6.2 Labeling 255
13.6.6.3 Nutritional Labeling 255

13.1 INTRODUCTION

All information in this chapter has been modified from several public documents available at the website of the U.S. Food and Drug Administration (FDA). The discussion resembles the format of teacher–student. The supervisor is describing the procedures to the inspection personnel. The objective of the presentation in this chapter is to assist the plant operation manager of a food company to become familiar with a food processing establishment inspection routinely conducted by the federal agency, FDA.

Food plant inspections are conducted to evaluate the methods, facilities, and controls used in manufacturing, storage, and distribution of foods. The general approach is as follows. Before undertaking an inspection some preparation should be carried out.

13.1.1 Preparation

1. Review the files of the firm to be inspected and acquaint yourself with the firm's history, related firms, trade marks, practices, and products. The review will identify products difficult to manufacture, that require special handling, special processes, or techniques, and hours of operation, which is especially important in bacteriological inspections. Remove, for subsequent investigations and discussion with management, Complaint/Injury Reports, which are marked for follow-up during the next inspection.
2. Become familiar with current programs relating to the particular food or industry involved.
3. Review and become familiar with the various parts of 21 CFR pertaining to foods, especially those of special significance to the firms being inspected.

13.1.2 Food Registration

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (the Bioterrorism Act) requires most domestic and foreign facilities that manufacture/process, pack, or hold food for human or animal consumption in the United States to register with the FDA. The Bioterrorism Act covers both interstate and intrastate firms. The regulations are codified at 21 CFR 1 Subpart H–Registration of Food Facilities. Registrations will be maintained in the FDA Unified Registration and Listing System (FURLS). Facilities are not considered to be registered until their information is entered into FURLS.

13.2 PERSONNEL

Obtain the full name and titles of the following individuals:

1. Owners, partners, or officers;
2. Other management officials or individuals supplying information;
3. Individuals refusing to supply information or permit inspection;
4. Individuals with whom inspectional findings were discussed or recommendations made.

Regulations require that plant management take all reasonable measures and precautions to assure control of communicable disease, employee cleanliness, appropriate training of key personnel, and compliance by all personnel with all requirements of FDA.

Determine if adequate supervision is provided for critical operations where violations are likely to occur if tasks are improperly performed.

13.2.1 Employees

Improper employee habits may contribute to violative practices in an otherwise satisfactory plant. Do the following:

1. Observe the attitude and actions of employees during all phases of the inspection.
2. Observe employees at their work stations and determine their duties or work functions.
3. Note whether employees are neatly and cleanly dressed and whether they wear head coverings that properly cover their hair.
4. Determine if employees working with the product have obvious colds, or infected sores, cuts, and so on. Under no circumstance should you swab a sore, touch, or remove a bandage from an employee in an attempt to obtain bacteriological data. To do so is a violation of personal privacy, possibly hazardous to you and/or the employee, and usually provides few useful data.
5. Note whether employees eat while on duty.
6. Observe and record insanitary employee practices or actions showing employees handling or touching unsanitized or dirty surfaces and then contacting food products or direct food-contact surfaces. Such practices might include employees spitting, handling garbage, placing their hands in or near their mouths, cleaning drains, handling dirty containers, and so on, and then handling food product without washing and sanitizing their hands.
7. Observe whether employees comply with plant rules such as, "No smoking", "Keep doors closed", "Wash hands before returning to work", and so on.
8. Be alert to employees handling insanitary objects, then quickly dipping their hands in sanitizing solutions without first washing them. Depending upon the amount and type of filth deposited on the hands during the handling of insanitary objects, such attempts at sanitizing are questionable at best. Sanitizers work most effectively on hands that have first been cleaned by washing with soap and water.
9. Conversations with employees doing work may provide information on both current and past objectionable practices, conditions, and circumstances. These should be recorded in your notes.
10. Where appropriate, determine employee education and training. Also determine type, duration, and adequacy of firm's training programs, if any, to prepare employees for their positions and to maintain their skills.

13.3 PLANTS AND GROUNDS

13.3.1 Environment

Observe the general nature of the neighborhood in which the firm is located. Environmental factors such as proximity to swamps, rivers, wharves, city dumps, and so on, may contribute to rodent, bird, insect, or other sanitation problems.

13.3.2 Plant Construction, Design, and Maintenance

Determine the approximate size and type of building housing the firm and whether it is suitable in size, construction, and design to facilitate maintenance and sanitary operations. Check placement of equipment, storage of materials, lighting, ventilation, and placement of partitions and screening to eliminate product contamination by bacteria, birds, vermin, and so on. Determine any construction defects or other conditions such as broken windows, cracked floor boards, sagging doors, and so on, which may permit animal entry or harborage.

Determine who is responsible for buildings and grounds maintenance. Many facilities such as docks, wharves, or other premises are owned and maintained by other firms, municipalities, or individuals for lease for manufacturing operations. Determine who is legally responsible for repairs, maintenance, rodent proofing, screening, and so on. Evaluate the firm's attitude toward maintenance and cleaning operations.

Inspect toilet facilities for cleanliness, adequate supplies of toilet paper, soap, towels, hot and cold water, and hand-washing signs. Check if hand-washing facilities are hidden, or if they are located where supervisory personnel can police hand washing.

13.3.3 Waste Disposal

Waste and garbage disposal poses a problem in all food plants depending upon plant location and municipal facilities available.

Check the effectiveness of waste disposal on the premises and ensure it does not cause violative conditions or contribute toward contamination of the finished products. Check for in-plant contamination of equipment and/or product, and whether its water is supplied from nearby streams, springs, lakes, or wells. Suspected dumping of sewage effluent into nearby streams, lakes, or bay waters near water intakes can be documented by color photographs and water-soluble fluorescein sodium of dye. Place approximately two ounces of dye, which yields a yellowish red color, into the firm's waste system and/or toilets, as applicable, and flush the system. The discharge area of the effluent becomes readily visible by a yellowish-red color on the surface of the water as the dye reaches it. Color photographs should be taken.

Determine collecting or flushing methods used to remove waste from operating areas. If water is used, determine if it is recirculated and thus may contaminate equipment or materials. Determine the disposition of waste materials that should not be used as human food, such as rancid nuts, juice from decomposed tomatoes. Determine the disposition of waste, garbage, and so on, that contain pesticide residues. Determine how this is segregated from waste material that contains no residues and that may be used for animal feed.

13.3.4 Plant Services

If applicable, check steam generators for capacity and demand. Demand may reach or exceed the rated capacity, which could effect adequacy of the process. Check boiler water additives if steam comes into direct contact with foods. Check central compressed air supply for effective removal of moisture (condensate) and oil. Determine if any undrained loops in the supply line exist where condensate can accumulate and become contaminated with foreign material or microorganisms.

13.4 RAW MATERIALS

General observations:

1. List in a general way the nature of raw materials on hand. Itemize and describe those that are unusual to you, or are involved in a suspected violation (copy quantity of contents and ingredient statements, codes, name of manufacturer or distributor, and so on).
2. Be alert for additives and preservatives.
3. Evaluate the storage of materials.
4. Determine the general storage pattern, stock rotation, and general housekeeping. Materials should be stored so they are accessible for inspection. Thoroughly check ceilings, walls, ledges, and floors in raw material storage areas for evidence of rodent of insect infestation, water dripping, or other adverse conditions.

Study the firm's handling procedure as follows:

1. Determine if growing conditions relative to disease, insects, and weather are affecting the raw material.
2. Check measures taken for protection against insect or rodent damage.
3. Raw materials may be susceptible to decomposition, bruising, or damage, for example, soft vegetables and fruits delivered in truckload lots. Determine the holding times of materials subject to progressive decomposition.
4. Evaluate the firm's acceptance examination and inspection practices, including washing and disposition of rejected lots. Where indicated, examine rejected lots and collect appropriate samples and report consignees.
5. Determine the general acceptability of raw materials for their intended use and their effect on the finished product. Raw stocks of fruits or vegetables may contribute decomposed or filthy material to the finished product.
6. Be alert for use of low-quality or salvage raw materials.
7. Check bags, bales, cases, and other types of raw material containers to determine signs of abnormal conditions, indicating presence of filthy, putrid, or decomposed items.
8. Check any indication of gnawed or otherwise damaged containers, to ascertain if material is violative.
9. Be alert to contamination of raw materials by infested or contaminated railroad cars or other carriers.
10. Document by photographs, exhibits, or sketches any instances where insanitary storage or handling conditions exist.
11. Determine whether the firm is aware of this publication and whether or not they comply.

13.5 EQUIPMENT AND UTENSILS

By arriving before processing begins, you are able to evaluate conditions and practices not otherwise observable before plant start-up. This includes adequacy of clean-up, where and how equipment is stored while not in use, how hand sanitizing solutions and food batches

are prepared, and if personnel sanitize their hands and equipment before beginning work. The following are some guidelines.

1. Dirty or improperly cleaned equipment and utensils may be the focal point for filth or bacterial contamination of the finished product. Examine all equipment for suitability and accessibility for cleaning. Determine if equipment is constructed or covered to protect contents from dust and environmental contamination. Open inspection ports to check inside only when this can be done safely. Notice whether inspection ports have been painted over or permanently sealed.
2. Observe the firm's filtering systems and evaluate the cleaning methods (or replacement intervals of disposable filters) and schedules. Check types of filters used. There have been instances where firms have relied on household-furnace-type-filters.
3. Check the sanitary condition of all machinery. Determine if equipment is cleaned prior to each use and the method of cleaning. If the firm rents or leases equipment on a short-term basis, report prior cleaning procedures. Equipment may have been used for pesticides, chemicals, drugs, and so on, prior to being installed, and could therefore be a source of cross-contamination.
4. Inspect conveyor belts for build-up of residual materials and pockets of residue in corners and under belts. Look in inspection ports and hard-to-reach places inside, around, underneath, and behind equipment and machinery for evidence of filth, insects, and/or rodent contamination. Chutes and conveyor ducts may appear satisfactory, but a rap on them with the heel of your hand or a rubber mallet may dislodge static material, which can be examined. Review procedure on taking in-line sample subs.
5. Determine how brushes, scrapers, brooms, and other items used during processing or on product contact surfaces are cleaned, sanitized, and stored. Evaluate the effectiveness of the practices observed.
6. Be alert for improper placement or inadequately protected mercury switches, mercury thermometers, or electric bulbs. Breakage of these could spray mercury and glass particles onto materials or into processing machinery.
7. If firm is using UV lamps for bacteria control, check if it has and uses any method or meters to check the strength of UV emissions. If so, obtain methods, procedures, type of equipment used, and schedule for replacement of weak UV bulbs.
8. In plants where chlorine solution is piped, check on the type of pipe used. Fiberglass reinforced epoxy pipe has been observed to erode inside through the action of the chlorine solution. This poses a threat of contamination from exposed glass fibers. Pipes made with polyester resin do not deteriorate from this solution.
9. Observe sanitizing practices throughout the plant and evaluate their effectiveness, degree of supervision exercised, strength, time, and methods of use of sanitizing agents. Determine the use, or absence of, sanitizing solutions both for sanitizing equipment and utensils as well as for hand dipping. If chlorine is used, 50–200 ppm should be used for equipment and utensils; a 100 ppm solution will suffice for hand-dipping solutions. Sanitizing solutions rapidly lose strength with the addition of organic material. The strength of the solution should be checked several times during the inspection.

13.6 MANUFACTURING PROCESS

Where helpful, to describe equipment and processes, draw flow plans or diagrams to show movement of materials through the plant. Generally, a brief description of each step in the process is sufficient. List all quality control activities for each step in the process and identify Critical Control Points. Provide a full description when necessary to describe and document objectionable conditions, or where the assignment specifically requests it.

Observe whether hands and equipment are washed or sanitized after contact with unsanitized surfaces, for example; in the following situations:

1. Workers do general work, then handle the product;
2. Containers contact the floor, then are nested or otherwise contact product or table surfaces;
3. Workers use common or dirty cloths or clothing for wiping hands;
4. Product falls on a dirty floor or a floor subject to outside foot traffic and is returned to the production line.

Be alert for optimum moisture, time, and temperature conditions conducive to bacterial growth. In industries where scrap portions of the product are reused or reworked into the process (e.g., candy and macaroni products), observe the methods used in the reworking and evaluate from a bacteriological standpoint. Reworking procedures such as soaking of macaroni or noodle scrap to soften, or hand kneading of scrap material, offers an excellent seeding medium for bacteria.

When a product is processed in a manner that destroys microorganisms, note whether there are any routes of recontamination from the "raw" to the processed product (e.g., dusts, common equipment, hands, flies, and so on).

13.6.1 Ingredient Handling

Use the following guide lines:

1. Observe the method of adding ingredients to the process. Filth may be added into the process stream from dust, rodent excreta pellets, debris, and so on, adhering to the surface of ingredient containers.
2. Evaluate the effectiveness of cleaning and inspectional operations performed on the materials prior to or while adding to the process.
3. Determine specific trimming or sorting operations on low-quality or questionable material.
4. Observe and report any significant lags during the process or between completion of final process and final shipping. For example, excessive delay between packing and freezing may be a factor in production of a violative product.

13.6.2 Formulas

Laws and regulations do not specifically require management to furnish formula information in food manufacturing, except for infant formulas. Nonetheless, they should be

requested particularly when necessary to document violations of standards, labeling, or color and food additives. Management may provide the qualitative formula but refuse the quantitative formula.

If formula information is refused, attempt to reconstruct the formula by observing:

1. Product in production;
2. Batch cards or formula sheets;
3. Raw materials and their location.

13.6.3 Food Additives

Be familiar with FDA's food additives program for instructions on conducting establishment inspections of firms manufacturing food additive chemicals. When making food plant inspections, direct your evaluation of food additives only to those instances of significant violation or gross misuse. Routine inspectional coverage will be directed primarily to the following two types of additives:

1. Unauthorized and illegal as listed in the FDA's Food Additives Status List (safrole, thiourea, and so on); and
2. Restricted as to amount in finished food.

Because of special problems, exclude the following additives from coverage during routine inspections:

1. Packaging materials;
2. Waxes and chemicals applied to fresh fruit and vegetables;
3. Synthetic flavors and flavoring components except those banned by regulations or policy statements (these products will be covered under other programs); and
4. Food additives in feeds (these products will be covered under other programs).

The Food Additives Status List (FASL) contains an alphabetical listing of substances that may be added directly to foods or feeds and their status under the Food Additives Amendment and Food Standards. In addition, a few unauthorized or illegal substances are included. Review this document. You may encounter substances not included in FASL. Such substances will include

1. Obviously safe substances not on the list of items generally recognized as safe (GRAS) that are not published in the regulations, for example, salt, cane sugar, corn syrup, vinegar;
2. Synthetic flavoring substances, because of their indefinite status;
3. Substances pending administrative determination;
4. Substances granted prior sanction for specific use prior to enactment of the Food Additives Amendment.

Give primary attention to unauthorized substances. Document and calculate levels of restricted-use additives in finished food only where gross misuse or program violations are suspected.

1. List ingredients, which may be restricted substances or food additives, and determine their status by referring to the current FASL. Report complete labeling on containers of these substances.
2. Obtain the quantitative formula for the finished product in question.
3. Determine the total batch weight by converting all ingredients to common units.
4. Calculate the theoretical levels in the final product of all restricted or unauthorized ingredients from the formula by using the Food Additives Nomographs.
5. Determine the probable level of restricted ingredients by observing the weight of each ingredient actually put into the batch.

13.6.4 Color Additives

Evaluate the status of all colors observed during each food establishment inspection by using FDA's Color Additives Status List (CASL). The list provides the current status and use limitations of most colors likely to be found in food, drug, device, or cosmetic establishments. Review the document.

Stocks of delisted and uncertified colors may be found in the possession of manufacturers where there is no evidence of misuse. Advise the firm of the status of these colors. If management wishes to voluntarily destroy such colors, witness the destruction. If the firm declines to destroy the colors, determine what disposition is planned, for example, use in nonfood products.

Where decertified or restricted-use colors are used in manufacturing food products, proceed as follows:

1. Collect an Official Sample consisting of the color and the article in which it is being used. Make every effort to collect interstate shipments of the adulterated product before attempting to develop a case for the court. When regulatory action is an alternative, obtain sufficient interstate records to cover both the color and the basic ingredients of the manufactured product. Refer to "Sampling Schedule for Color Containing Products" for guidance.
2. Document the use of decertified colors after the decertifying date. Documentation should include batch formula cards, employee statements, code marks indicating date of manufacture, color certification number, and so on. The presence of color in the finished product will be confirmed by your servicing laboratory.

13.6.5 Quality Control

The objective of quality control is to ensure the maintenance of proper standards in manufactured goods, especially by periodic random inspection of the product. Your inspection should determine if the firm's quality control system accomplishes its intended purpose. Establish responsibility for specific operations in the control system. Determine which controls are critical for the safety of the finished product.

13.6.5.1 Inspection System

1. Determine what inspectional control is exercised over both raw materials and the processing steps. Such inspection may vary from simple visual or other organoleptic examination to elaborate mechanical manipulation.
2. Determine what inspection equipment is used, that is, inspection belts, sorting belts, grading tables, ultraviolet lights, and so on. Ascertain its effectiveness, maintenance, or adjustment schedules.
3. Where indicated, determine the name of the manufacturer of any mechanical inspection device and the principles of its operation.
4. Evaluate the effectiveness of the personnel assigned to inspection operations. Determine if the inspection belts or pick-out stations are adequately staffed and supervised.
5. Determine the disposition of waste materials that are unfit for food or feed purposes.

13.6.5.2 Laboratory Tests

1. Describe routine tests or examinations performed by the firm's laboratory and the records maintained by the firm.
2. Determine what equipment is available in the laboratory and if it is adequate for the purpose intended. If the firm uses a consulting laboratory, determine what tests are performed and how often.
3. Review laboratory records for the period immediately preceding the inspection.

13.6.5.3 Manufacturing Code System

1. Obtain a complete description of the coding system with any necessary keys for interpretation.
2. Provide an example by illustrating the code being used at the time of the inspection (see 21 CFR 113.60(c) and 114.80(b)).
3. Report coding systems that require the use of UV light for visibility.
4. Hermetically sealed containers of low-acid processed food must be coded in a manner clearly visible (see 21 CFR 113.60).
5. Check 21 CFR 113 and 114 for regulations on coding for the type of plant you are inspecting.

13.6.6 Packaging and Labeling

1. Evaluate storage of packaging materials including protection from contamination by rodents, insects, toxic chemicals, or other materials.
2. Appraise the manner in which containers are handled and delivered to the filling areas.
3. Determine if there is likelihood of chipping of glass or denting, puncturing, tearing, and so on, of packaging materials.
4. Observe the preparation of containers prior to filling. Consider any washing, steaming, or other cleaning process for effectiveness.
5. Determine, in detail, the use of air pressure or other cleaning devices.

13.6.6.1 Quantity of Contents. If slack fill is suspected, weigh a representative number of finished packages. See guidelines for net weight procedure. Sets of official weights are available in the servicing laboratory. These may be used to check the accuracy of a firm's weighing equipment.

13.6.6.2 Labeling

1. Check the sanitary condition of labelers and equipment feeding cans to, and away from, the labeler. Determine if old product is present on any equipment that touches the can end seams, in the presence of moisture carryover from the can cooling operation.
2. Check availability of floor drains in the labeling area. Absence of floor drains could indicate infrequent cleaning of the equipment unless it is physically moved to another area for cleaning.
3. Determine what labels are used and what labeling is prepared or used to accompany or promote the product. Obtain specimens of representative labels and labeling including pamphlets, booklets, and other promotional material.

13.6.6.3 Nutritional Labeling. See document "Guide to Nutritional Labeling and Education Act (NLEA) Requirements" for guidance.

Part B

Food Products Manufacturing

This Part includes Sections, IV to XVII.

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

Section IV

Bakery Products

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

14

Manufacturing of Bread and Bakery Products

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14.1 Introduction 262
14.2 Product Characteristics 263
14.2.1 Bread 263
14.2.2 Cake 263
14.2.3 Biscuit 264
14.2.4 Others 264
14.3 Ingredients and Their Impact on Product Quality 265
14.3.1 Flour 265
14.3.2 Water 265
14.3.3 Fat 266
14.3.4 Sugar 266
14.3.5 Salt 266
14.4 Processing Steps 266
14.4.1 Dough Making 267
14.4.2 Proving 268
14.4.3 Baking 268
14.4.4 Cooling 270

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

14.5 Packaging and Shelf-life 270
14.6 Process Modeling for Technology Improvement 272
14.7 Current Trends 273
14.8 Summary 274
References 274
Websites 278

14.1 INTRODUCTION

In the food manufacturing industries, the baking industry is one of the most stable sectors (Liberopoulos and Tsarouhas 2005). Bread and bakery product development has a long history. The most recent archeological discovery indicated that baking might date back to as early as around 21,000 B.C. (Piperno and others 2004), when people knew wheat and learned to mix wheatgrain meal with water and bake it on stones heated by fire – the first flat bread. During the period 2600 to 3000 B.C., yeast was used by ancient Egyptians to make fermented bread. Due to the high cost of milling, wholewheat and wholegrain bread was generally consumed. White bread was also available, but limited only to groups of rich people. Since the 1900s, white bread has become common for everyone. However, there has recently been a reverse trend, where wholewheat and wholegrain bread has become more expensive than white bread, due to health concerns. Meanwhile, technological advances have enabled wholemeal bread to be developed to become both nutritious and tasty (www.breadinfo.com/history.shtml). Cake, meanwhile, has been developed since the discovery of flour. At the beginning, yeast was used as a raising agent for cake. It was then replaced by eggs in the middle of the eighteenth century (whatscookingamerica.net/History/CakeHistory.htm; www.themediadrome.com/content/articles/food_articles/cake_ history.htm). Conventionally, cake was defined as a flour-based sweet food, whereas bread was defined as flour-based nonsweet food. In addition, the word "cake" was used for small bread. Cake was normally made from expensive ingredients, therefore it was specially made for certain occasions (whatscookingamerica.net/History/CakeHistory.htm). From culinary historians, we learn that cake batter was once used for the purpose of oven temperature testing. As a result, a new product was created and called "biscuit" (in the United Kingdom) or "cookie" (in the United States). That is why biscuit was originally described as a flour-based sweet cake. It can be either crispy or soft. Around 1600, people started traveling to discover the world. As biscuit has a much longer shelf-life, it was ideal for use as a food for travelers (whatscookingamerica.net/History/CookieHistory.htm).

In earlier days, the bakery business started in small bakeries with large varieties of products in every village. Later, the small bakeries were replaced with industrial mono-production, particularly for bread. This was mainly because bread could then be produced efficiently. Therefore, the key for success is productivity (Decock and Cappelle 2005). Typically, a modern-day production line is composed of several workstations integrated into an automatic system using common transfer mechanisms and control systems. The nominal production rate can be determined from the bottleneck workstation, which is almost always a baking oven (Liberopoulos and Tsarouhas 2005). Decock and Cappelle (2005) found that the consequence of evolution of the bakery business was a decreased interest in bread flavor from a long fermentation process. In contrast, artisan bakeries and specialty bread from all over the world became more interesting to consumers. Moreover, issues related to health have become

more of a concern. The effect of information on dietary fiber has been studied. In Australia, it was found that the likelihood of the consumption of white products including bread and English muffins could be enhanced by a label claiming "high in fiber" (Mialon and others 2002). According to an annual survey of manufacturers in 2001, the total value of the manufacturing of bread and bakery products in the United States was US$31.2 billion including retail bakeries (US$2.5 billion), commercial bakeries (US$25.7 billion), and frozen products and other pastries (US$3.0 billion) (Liberopoulos and Tsarouhas 2005). Meanwhile, according to the Euromonitor International sources, the total global market value of bread and bakery products stood at US$311.1 billion in 2005, representing a 7.32% increase from 2004.

14.2 PRODUCT CHARACTERISTICS

14.2.1 Bread

Bread is a product consisting of two distinctly different parts: crust and crumb. Crust is a dry and crispy surface layer, and the crumb inside bread loaves or buns is soft. To produce a soft and fine crumb, bread dough requires the proper development of a gluten network during dough mixing. Varieties of bread differ in size, shape, color, texture, and flavor. They contain different ingredients and may also be baked under significantly different conditions. Bread can either be broadly classified as leavened and unleavened, depending on the present or absence of leavening agents in dough, or be classified as yeast bread and quick bread (Zhou and Therdthai 2006).

Sourdough bread is a traditional bread with natural flavor developed by yeast (commercially *Saccharomyces cerevisiae*) and lactic acid bacteria (mostly *Lactobacillus* genus). It has an acidic sharp taste. Compared to normal bread, the addition of lactic acid bacteria can slow down the staling rate during bread storage (Gul and others 2005). The ratio of lactic acid to acetic acid is also an important factor. Corsetti and others (1998) found that a high ratio of lactic acid to acetic acid could reduce the staling rate as well as increase volume expansion. To obtain the highest quality sourdough, the interaction between yeast and lactic acid bacteria therefore has to be clearly understood. Gul and others (2005) found that a mixture of 1.5% *Saccharomyces cerevisiae* and 1.5% *Lactobacillus amylophilus* could produce dough and bread with the best quality attributes including yield, specific volume, and harness. However, a sensory evaluation revealed that consumers preferred sourdough bread made from a mixture of 1.5% *Saccharomyces cerevisiae* and 1.5% *Lactobacillus sake*.

14.2.2 Cake

Cake is made by the formation of a batter, which is a complex emulsion and foam system with an appreciable amount of bubbles inside. The required ingredients are similar to bread dough, except weak wheat flour is needed. A gluten network is not required for cake because it would make the cake firmer and chewier. The ratio of water to flour to make a cake batter is high, compared to that in bread dough. Therefore, one of the important rheological properties of cake batter is its low viscosity.

Eggs are normally among the ingredients for cake, and the egg protein acts as a surface active agent to form a protective film around air bubbles. This helps to keep the air bubbles in the batter during mixing. The mixing process is ended when there are enough air bubbles kept in the batter and the density of the batter reaches its minimum. To keep the product fresh for longer, oil or fat solid is needed. With the addition of oil/fat, the

batter becomes an oil-in-water emulsion system. Due to the positive effect of soluble solids on the starch gelatinization temperature, the ratio of water to soluble solids, especially sucrose, is one of the key factors for cake making. Recently, computer programs have been introduced for designing balanced ingredients to create different cake recipes (Cauvain and Young 2000).

14.2.3 Biscuit

Biscuit is a low-moisture bakery product. According to the information provided by various manufacturers, its moisture content varies widely, because of the variation in its thickness and weight during forming and shaping (Cronin and Preis 2000). Typically, the moisture content of biscuit after baking is below 10%. Therefore, water addition to biscuit dough is lower compared to other bakery products. The size of the biscuit after baking is affected by the rheological properties of the dough. For sweet biscuit that contains high levels of sugar and fat, the dough has good cohesiveness and plasticity without the formation of a gluten network (Gallagher and others 2003a). Therefore, short dough can be made from soft wheat flour with a high amount of sugar (25–55%) and shortening (20–60%) (Baltsavias and others 1999).

Using the preference mapping technique, Martinez and others (2002) found that four characteristics are mainly responsible for the consumer acceptance of the cracker type of biscuits, including appearance, aroma and flavor, texture and overall acceptability.

A typical defect of biscuit is cracking or checking, as a result of the moisture gradient in the product after baking. The moisture content of freshly baked biscuit is high in the center and low at the edge. Therefore, moisture migrates from the centre to the edge, which causes an expansion at the edge, but a contraction at the centre. Subsequently, stress is developed and when it exceeds a critical level, crack may occur (Saleem and others 2005).

14.2.4 Others

Besides the major bakery products described above, there are also numerous other types of bakery products, including pastries, pizzas, breakfast cereals, and so on. Other than wheat flour, many bakery products are also made from other cereal flours, such as barley, rye, corn, soy, rice, and oats.

Pastry products are characteristically flaky, tender, crisp, and slightly browned. Pastry can be classified into two types: plain pastry and puff pastry. Plain pastry is used mainly for pie crust, and puff pastry is primarily found in desserts such as Napoleons, patty shells, tarts, and cream horns. The proportion of fat is probably the most important determinant of quality in pastry, especially in creating flakiness. As little water as possible should be added to the pastry dough, because too much water will cause shrinkage and a tougher crust from excess gluten development (Brown 2004).

Breakfast cereals in today's context are ready-to-eat (RTE) cereals. They are cereal grains processed in ways that make them suitable for human consumption. They are made from various grains and in many forms, including flakes, shredded shapes, gun puffed shapes, extruded shapes, and so on. The products are often fortified with vitamins and minerals. In making breakfast cereals, slurry, rather than dough, is often made first by blending the dry ingredients with liquid ingredients, followed by cooking, drying/roasting (i.e., baking), tempering, and forming (Fast 2001). It is worth noting that baking might not be part of the process for making some breakfast cereals, therefore it may not be appropriate to classify all breakfast cereals as bakery products.

14.3 INGREDIENTS AND THEIR IMPACT ON PRODUCT QUALITY

14.3.1 Flour

The major constituents of wheat flour are starch (65–75%) and proteins (7–16%). Starch consists of mainly amylose and amylopectin. In dough, starch is present as semicrystalline granules that absorb a significant amount of water and may act as filler in the continuous protein network, or may form a complex network with the proteins. During baking, the starch granules gelatinize, which is a major part of the solidification process of the dough. The gelatinization starts at 55°C and finishes at 85°C when water evaporation at the surface dominates (Engelsen and others 2001). A series of processes at a molecular scale include swelling, melting, disruption of starch granules, and exudation of amylose. In bread, as a result of starch gelatinization, the partially swollen granules can be stretched into elongated forms to allow gas cells to expand. Therefore, texture and structure of the product is dependent on starch gelatinization (Therdthai and Zhou 2003).

Quality of flour in terms of protein content has been found to be a key factor influencing bakery product quality. Wheat flour contains some specific proteins such as glutenins, and gliadins, which can absorb water and form gluten. Sliwinski and others (2004) found a positive correlation between gluten, content and bread volume, due to the gluten's key role in building up a dough structure to retain gas inside the dough. Gas retention capacity varies depending on the rheological properties of dough, such as viscosity and extensibility (Kokelaar and others 1996). For uniaxial extension, wheat flour with higher protein content had higher elongation extensibility. High dough viscosity could prevent ascent of gas cells, whereas high extensibility could prevent coalescence and thereby premature fracture of membranes between gas cells. With high dough strength, the expansion of gas cells during proving and baking could be limited (Sliwinski and others 2004). Freeze-dried gluten could increase water addition, dough resistance, and water-holding capacity, as well as reduce the firmness of the bread bun. Wet, freshly extracted gluten could improve the texture of bread, but the extraction needs to be carried out every time the gluten is needed, which is necessary for keeping the gluten's good functional characteristics including the rate of water absorption and the degree of viscoelasticity (Esteller and others 2005).

14.3.2 Water

Water is one of the main ingredients of bakery products. It influences the formation of dough and batter and therefore the textural properties of baked products. Water is needed for hydrating the proteins, therefore developing the gluten, gelatinizing the starch, making the leavening agents function, activating the enzymes, dissolving sugar and salt, as well as acting as a major heat transfer mechanism during bread baking through evaporation and condensation.

The optimum amount of water addition is dependant on the required viscosity and product characteristics. Sliwinski and others (2004) stated that puff pastry dough requires lower water addition than bread dough. For baguette, high water addition is needed together with soft dough consistency to develop an open cell structure. In general, too little water addition leads to high viscosity, therefore the dough shape is not flexible during dividing and molding. On the other hand, too much water makes viscosity low, and dough might not be able to retain its shape during proving. However, the problem is not significant with pan-proving dough. In addition to product characteristics, dough-making process also varies the requirement on water addition. For a bread dough-making process with 1 h bulk

fermentation, 57% water may be added. When the fermentation becomes longer, at 4 h, the water addition should be reduced to 55%. For the no-time dough making in the Chorleywood Breadmaking Process (CBP), water can be added up to 60%. Extra 2% water may be added when partial vacuum is applied during mixing (Cauvain and Young 2000).

14.3.3 Fat

Fat functions to reduce dough viscosity and relaxation time, through coating the flour particles and preventing the formation of gluten. This provides a shortness character to the products, that is, a soft, pleasant, and crumbly texture.

Biscuit with a high fat content has a friable structure (Maache-Rezzoug and others 1998a). In addition, Chevallier and others (2000) stated that fat could delay the effect of chemical leavening agents. In cake batter, addition of oil or fat decreases the foam-stabilizing properties of egg and hydration of flour protein (Cauvain and Young 2000). To reduce the fat content in bakery products, protein-based fat replacers (e.g., Simplesse®) can be used (Gallagher and others 2003a).

14.3.4 Sugar

Similarly to fat, sugar also functions to reduce dough viscosity and relaxation time (Maache-Rezzoug and others 1998a), as well as to delay the effect of chemical leavening agents during baking (Chevallier and others 2000). Biscuit with a high sugar content contains a cohesive structure and crisp texture (Maache-Rezzoug and others 1998a). Moreover, sugar could increase the temperature of the starch phase transition (Chevallier and others 2000). To reduce the sugar content in bakery products, sugar replacers might be used. Gallagher and others (2003b) found that fructooligosaccharide could replace sugar at low (20%) and medium levels (25%) but still maintain the snapping characteristics of short dough biscuits.

14.3.5 Salt

Salt is added into dough not only to be a seasoning or flavor enhancer, but also to strengthen the gluten structure. However, it also inhibits yeast growth. It can be used for controlling fermentation (Gisslen 1994). Gianotti and others (1997) found that salt could be used to control the growth rate of both lactic acid bacteria and yeast. Increasing the salt content can slow down the fermentation, therefore the proving process, whereas decreasing the salt content may cause an overfermentation. In the case of overfermentation, a large amount of sugar would have been used for the yeast growth. As a result, the crust color may not be brown enough (Gisslen 1994).

14.4 PROCESSING STEPS

Generally, the production of bread and bakery products consists of several common steps, including weighing, mixing, proving, baking, cooling, and packaging. Each step plays an important role in achieving high and consistent product quality. In industrial bakeries, flour is stored in silos and transferred to production lines. In production lines, flour and other ingredients are mixed and kneaded to make dough or batter. Then the dough/batter is divided and molded. Some of the bakery products require a fermentation step to develop unique flavor and/or texture prior to the baking step at 180–300°C. The products are then cooled down before packing and delivery. Baking trays and molds are normally cleaned in hot water at 40°C (Kannan and Boie 2003).

14.4.1 Dough Making

All ingredients, including mainly flour, water, leavening agent, fat, and sugar, are weighed in hoppers before being released sequentially into a mixer. Mixing is a process that uses the mechanical action of kneaders and the corresponding energy to mix all ingredients to form a coherent material, that is, dough. There are several dough-making techniques used to modify the rheological characteristics of dough to optimize its gas-retaining properties (Cauvain 1998). During mixing, there is a gradual uptake of water by flour (composed mainly of protein and starch), that is, hydration. During hydration, the protein changes from a hard glassy material to a soft rubbery material through a glass transition. At room temperature (20°C), the glass transition for gluten occurs at about 16% moisture content. The moisture content at which the glass transition occurs increases when temperature decreases. However, it is still within the typical range of the water to flour ratio for breadmaking. Decreasing the dough temperature at the end of mixing produces a firmer dough with less oxidation. Some additional water is needed to adjust the dough consistency. Increasing the dough temperature would speed up the enzymatic activity, yeast fermentation, and proving rate. Moreover, the dough texture becomes softer and less resistant to deformation. A dough-making process with a period of bulk fermentation typically has a dough temperature of 23–26°C, whereas a no-time dough-making process may have a dough temperature of 28–31°C. In order to obtain a suitable dough temperature at the end of mixing, the temperature of the added water has to be controlled (Cauvain and Young 2000).

To increase the rheological characteristics of dough, including elastic recovery, cohesiveness, and/or adhesiveness, the mixing time should be extended. In contrast, the apparent biaxial extensional viscosity and consistency can be increased by shortening the mixing time. According to Sliwinski and others (2004), increasing the mixing time could increase the volume of puff pastry. However, there was no correlation between the mixing time and product volume in the case of bread. For biscuit, increasing the mixing time could soften the dough, reduce the relaxation time, increase the product length, and decrease the product weight (Maache-Rezzoug and others 1998b). Manohar and Rao (1997) found that a long mixing time of 300 s produced biscuit with a dense structure, low crispness, and high breaking and compression strength values. However, a short mixing time of 90 s resulted in a wrinkled surface. Compared to the mixings of 300 s and 90 s, a mixing time of 180 s produced a superior biscuit.

In addition to mixing time, the sequence of ingredients to mix is also important. The arrangement of the sequence may affect the flour hydration behavior and therefore the dough texture. In bread, Cauvain and Young (2000) suggested that salt should be added later in mixing, particularly when a low-speed mixer is used, because salt reduces the amount of water available to hydration and gluten development. In biscuit, the creaming technique (i.e., all ingredients excluding flour and nuts are mixed together first) is used to make a cream mixture for blending with flour at the end of the mixing process. This results in significantly less gluten development, and therefore a soft dough. Maache-Rezzoug and others (1998b) found that dough could be softened by mixing sugar with liquid ingredients before mixing with other dry ingredients, because sugar prevented the hydration of the flour constituents by reducing the water activity of the dough during mixing and therefore inhibited gluten formation.

In the CBP, where the mixing is carried out using a fixed amount of energy input, partial vacuum can be applied towards the end of mixing in order to increase dough water addition. As vacuum makes a dough firmer and drier, extra water is required to maintain the dough consistency (Cauvain and Young 2000). Moreover, the applied vacuum may decrease the average size of bubbles (Cauvain 1998) and the amount of bubble gas by 50% (Marsh 1998) in the dough. Figure 14.1 shows the interior of a Tweedy mixer that is typically used in the CBP.

Figure 14.1 *Interior of a Tweedy mixer (as typically used in the CBP). (Courtesy of APV Baker.)*

14.4.2 Proving

Proving is an important step for bread and other yeasted bakery products that use yeast to produce carbon dioxide through fermentation to increase the dough volume. After the dough-making step, dough is normally proved at 38°C, as yeast is most active within the temperature range of 25–40°C. Proving time is dependent on the yeast level, proving temperature, and humidity. Increasing the yeast level is likely to speed up the proving process (Anon. 1995). Yeast uses sugar as a carbon source for growth. However, too much sugar will damage yeast by an osmotic dehydration mechanism. This is why volume expansion is always a problem for sweet bread bun. Similarly, salt can inhibit yeast growth as described earlier, and too much salt will slow down the proving process. Generally, the yeast used in bakery products is *Saccharomyces cerevisiae*. Various yeast strains have been developed to have different tolerances to osmotic dehydration and therefore suit different types of bread (Linko and others 1997). To achieve the correct relative humidity, steam or hot water can be used to increase the humidity in a proving chamber in order to speed up the process.

14.4.3 Baking

Baking is a complex unit operation involving simultaneous heat and mass transfer mechanisms. Heat is supplied to the dough through radiation, convection, and conduction. Thermal reactions including starch gelatinization and protein denaturation are activated. When temperature increases and protein is denatured, water originally held in the protein network during proving is released. Starch uses this water to form a gel. The physicochemical properties of dough are changed and, as a result, dough is changed and, to a baked product with a porous and rigid structure.

The volume expansion of bakery products during baking is through two different mechanisms. Taking bread as an example, at the early stage of a baking process, the dough volume is expanded mainly due to yeast fermentation. When temperature is increased to around 55°C, yeast would be killed. Once the temperature reaches 60°C, any volume expansion is mainly due to vapor pressure (Fan and others 1999). During baking, heat

from the oven penetrates to the product core by the combined mechanism of conduction, water vapor evaporation, diffusion, and condensation, and water migrates from the product core to the surface by combined evaporation, vapor diffusion, and condensation, as well as liquid diffusion (Therdthai and Zhou 2003). The product volume keeps increasing until a rigid structure is formed and restricts the expansion. The developed crust becomes the main resistance to heat and mass transfer. At the surface of the dry crust, water vapor is transferred by convection under the gradient of pressure (Lostie and others 2002).

In addition to textural and structural changes, color and flavor are developed during baking. When the surface temperature reaches 150°C, browning reactions are activated and therefore the crust color becomes darker. For flavor, volatile compounds are formed and released, which gives the bakery products a uniquely good smell after baking.

To maintain product uniformity and quality consistency, it is necessary to adjust the oven operation from time to time. Such adjustments result in higher cost in terms of time and raw materials. Several researches have been conducted to develop reliable models for simulating the various phenomena during baking (Therdthai and others 2003, 2004a). The simulation can predict the consequence of any change in the operating condition, which may be used to establish the optimal operation conditions for different product varieties. For designing effective process control systems, on-line sensors are needed to collect data and inform a controller in order to regulate the baking condition at a set level. For biscuit baking, color can be monitored from the top of biscuits using a Colorex sensor. The reflection of a light source (D65) is analyzed and converted into an electrical signal (Perrot and others 1996).

A conventional baking oven typically consists of baking chambers and heating elements. Heat is transferred, either directly or indirectly, to dough/bread, through radiation, convection, and conduction. Figure 14.2 shows a direct heating oven. However, new baking ovens have been developed to improve product quality as well as process efficiency. For example, microwave has been introduced to baking ovens to speed up the baking process. Dough is heated up rapidly by the movement of water molecules under the influence of the microwaves. Therefore, the ambient temperature of a microwave baking oven is lower than that of a conventional baking oven. Not only can it save energy, but it also helps to maintain the nutritional value of the baked products. However, surface color might not be fully developed, because the surface temperature is too low and the baking time is too short to complete

Figure 14.2 *A direct heating over. (Courtesy of C. H. Babb Co., Inc.)*

the Maillard browning reactions. Similar problems can occur with starch gelatinization and flavor development (Sumnu 2001). However, these problems may be reduced by integrating conventional baking ovens with microwave (Verboven and others 2003). Recently, impingement ovens have been introduced to industrial baking. Due to the very high velocity in the oven, baking time can be significantly shortened. However, product loss tends to be increased as well (Wählby and others 2000).

14.4.4 Cooling

Baked products have to be cooled down to room temperature in order to avoid condensation after packaging. The most common method for cooling is natural cooling using room air or chilled air. Figure 14.3 shows a natural cooling system. A cooling process can take from a few minutes to a few hours depending on the product characteristics and cooling methods. Using room air, some products such as panetonni, a fermented Italian cake, may need 24 h for cooling. To shorten the cooling process, a vacuum cooler can be used, which cools panetonni within 4 min. This also applies to some other products such as breadsticks, meat pies, sausage rolls, pastries, and cakes, which can be cooled in between 1 and 3 h by conventional cooling, but within only 30 s to 5 min by vacuum cooling (Anon. 1978). With regard to weight loss during cooling, Everington (1993) found 6.8% loss after cooling from 98°C to 30°C by vacuum cooling, but cooling using an air blast cooler caused just 3–5% loss. However, vacuum cooling can cause structural changes due to the build-up of vapor pressure in areas of low vapor permeability. Modulated vacuum coolers may be used to alleviate this problem by modulating pressure, instead of continuous pressure, resulting in controlled vacuum during cooling (Sun and Zheng 2006).

14.5 PACKAGING AND SHELF-LIFE

In terms of shelf-life, bakery products can be divided into two groups: low- and intermediate-moisture products, and high moisture products. Normally, the shelf-life of

Figure 14.3 *A natural cooling system. (Courtesy of Baking Technology Systems, Inc.)*

low- and intermediate-moisture products is limited by physical or chemical spoilage (Smith and others 2004). For low-moisture products such as biscuits, moisture absorption is the main problem, resulting in a product texture changed from crispy to soft. It is affected by the equilibrium of water activity and mass transfer mechanism (Labuza and Hyman 1998). For intermediate moisture products such as bread and cake, the physico-chemical change is normally associated with starch retrogradation. Water distribution among the high-molecular-weight substances such as protein and starch causes the water to transform from strongly bound to weakly bound. Therefore, staling can be observed (Schiraldi and others 1996). Baeva and Panchev (2005) found 16% reduction of bound water in a dietetic sucrose-free cake after storage for 6 days.

The shelf-life of high-moisture products is limited by microbiological spoilage, mainly by yeast and mold. Moreover, some foodborne microbes including *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus* are of concern (Smith and others 2004). Bailey and von Holy (1993) studied *Bacillus* spore contamination on a brown bread production line in a commercial bakery in South Africa. High counts of *Bacillus* spores were obtained mainly from yeast, crumbs of broken loaves, and premix. The spore counts after mixing, baking, and 3 days storage was 2.6 log cfu/g, 2.1 log cfu/g, and 6.4 log cfu/g, respectively. To detect spoilage before it becomes visible, an electronic nose can be applied, which as differentiate spoilage by microorganisms from that by lipoxygenase enzymes (Needham and others 2005).

To prolong the shelf-life of bakery products, modified atmosphere package (MAP) can be used. A combination of gases plays the most important role in maintaining product quality during MAP storage. To prevent the growth of mold and provide the greatest stagnancy, carbon dioxide and nitrogen gases should be used in ranges of 20–50% and 80–50%, respectively (Kotsianis and others 2002). However, spoilage by *Clostridium botulinum* has to be of concern when MAP is used (Smith and others 2004).

In addition to MAP, edible films can be used to cover products. Baeva and Panchev (2005) used a pectin-containing edible film to cover a dietetic sucrose-free cake. The uncovered cake exhibited quicker crumb aging compared to a sucrose-sweetened cake. After storage for 6 days, the cake covered with the film had 1.5 times higher amount of strongly bound water, compared with the uncovered cake. This indicated a slower rate of retrogradation, therefore slower aging, in the film-covered cake. Moreover, shrinkage was found after 5 days of storage, and slight changes in crumb yellow color were also observed.

Utilization of preservatives is another method to prolong the shelf-life of bakery products. To prevent fungi spoilage in intermediate-moisture products, weak acid preservatives, especially propionic, sorbic, and benzoic acids, can be used. Propionic acid is used for the inhibition of mold and *Bacillus* spores. Sorbic acid is used for the inhibition of mold and yeast. It can be used in combination with benzoic acid. Due to a better solubility in aqueous solutions, the preservative acids are used normally in the form of salts.

Acidity plays an important role in the effectiveness of preservatives. When products have high acidity (pH 4.4–4.8) and high water activity (a_w, 0.94–0.97), 0.3% propionate can inhibit fungal growth (except *Pencillium roqueforti*, *Penicillium commune*, and *Eurotium rubrum*) for 2 weeks (Suhr and Nielsen 2004). Guynot and others (2005a) utilized the hurdle technology (hurdle technology is on combining several preservation techniques of mild degree to make the overall process effective to ensure microbiological safety and stability of a food), including weak acid preservatives (0–0.3%), water activity (0.80–0.90), and pH (4.5–5.5) for the inhibition of mold. It was found that 0.3%

potassium sorbate was effective at 0.90 water activity and pH 4.5. However, it became less effective at pH 5.5. To make calcium propionate and sodium benzoate effective, water activity had to be kept at a low level (0.80). The relationship between fungal growth and storage parameters including potassium sorbate concentration, water activity, and pH could be described by polynomial models having 10 terms (Guynot and others 2005b). At higher water activity (a_w, 0.93–0.97), combining potassium sorbate with calcium propionate could be used to inhibit fungal growth when the acidity was kept at 4.5 (Arroyo and others 2005).

At higher pH (6.0 and above), potassium sorbate and sodium benzoate were more effective than calcium propionate (Suhr and Nielsen 2004). Among the weak acid preservatives, potassium sorbate was the most effective (Guynot and others 2002). However, at near neutral pH, 0.1% sorbate presented little antifungal effect. When the concentration was increased to 0.2%, sorbate could inhibit the growth of mold effectively under some conditions of water activity and storage temperature (Marin and others 2003). Guynot and others (2002) stated that 0.3% sorbate was effective under the conditions pH 6.0 and an a_w of 0.80–0.85.

For bakery products with an unbaked filling, contamination from equipment could be very easy. To delay the growth rate of pathogenic microorganisms, the pH of the filling has to be reduced by addition of acids such as acetic acid, citric acid, and so on. Bakery products with a filling made of fruits are possibly more stable because of their lower pH (Leitenberger and Rocken 1998).

Without preservatives, water activity and storage temperature have to be optimized to prevent any fungal growth in intermediate moisture products. In the case of a sponge cake, Abellana and others (2001) concluded that the water activity had to be lower than 0.85 for storing the product at a temperature above 15°C.

14.6 PROCESS MODELING FOR TECHNOLOGY IMPROVEMENT

Modeling has been carried out for describing all events throughout the bakery process. For product formulation, the impact of ingredients on product quality can be expressed by mathematical models. Based on the models, various recipes can be designed (Young and others 1998). Furthermore, a Convected Maxwell Model was used to simulate the stress–time curves during mixing (Maache-Rezzoug and others 1998b). During baking, heat distribution has been described using mathematical models (Fahloul and others 1995; Sablani and others 1998) and computational fluid dynamics (CFD) (De Vries and others 1995; Therdthai and others 2003, 2004a). Using artificial neural networks, the thermal conductivity of bakery products can be modeled as a function of moisture content, temperature, and apparent density (Sablani and others 2002). Moisture diffusion and transfer in biscuits during baking can be simulated using mathematical models based on Fick's second law (Guillard and others 2004). The effect of baking profiles on the quality (Therdthai and others 2002) and cost (Dingstad and others 2004) of bread may be described using empirical models. Variations in material properties can be used to simulate cracking in biscuits using finite element modeling (Saleem and others 2005). Reactions in bread such as gelatinization and browning have been estimated using first-order kinetic models (Zanoni and others 1995a, 1995b). To facilitate designing the optimal operating condition for a bread-baking process, several models could be integrated to predict the product quality resulting from various baking conditions or process

modifications (Therdthai and others 2004b). During cooling, the effect of air temperature, initial product temperature, and air velocity on the cooling rate should be evaluated, and empirical models can be established directly based on the industrial data. As described earlier, the shelf-life of intermediate-moisture bakery products after packaging could be estimated using polynomial models with 10 terms (Guynot and others 2005b). Patterns in the product acceptance of biscuit crackers have been studied using the mapping technique (Martinez and others 2002).

Recently there have been several commercial computing programs introduced to the baking industry, such as the CCFRA Cake Expert System by the Campden and Chorleywood Food Research Association (CCFRA), Food Processor by ESHA Research, Balance by CCFRA, and SolidWorks ® by SolidWorks Corporation (Otles and Onal 2004). These software all use modeling approaches to compute/design the optimum formulation and process. Such an approach can improve bakery manufacturing with an economic design of product and process.

14.7 CURRENT TRENDS

As mentioned earlier in the introduction section, the rising health consciousness of today's consumers has been constantly shaping trends in bakery products. Although the low-carb craziness might well be over, other issues such as high-fiber multigrains have been among the major market drivers. Well in line with the global functional food trend, new varieties of bread products, sometimes called functional breads, have been developed and released, particularly those enriched or fortified with particular grains or other ingredients (Anon. 2006).

Recently, there has been considerable interest in plant polyphenols and phytosterols, as many of them are strong antioxidants and/or cholesterol-reducing substances. Incorporating these active components into traditional bakery products would turn the products into modern functional food items. However, recent studies on incorporating tea catechins into bread have revealed that, although the tea antioxidants might be stable enough so that most of them can survive the bread-making process, they may impose significant negative effects on the quality of bread in terms of volume and texture, depending on the dose level (Wang and Zhou 2004; Wang and others 2006). In another study, phytosterol esters have been incorporated into croissants and muffins, together with α-tocopherol and β-carotene, and the products were given to normocholesterolemic and habitual consumers, respectively, to provide 3.2 g/day of the phytosterol esters. It was found that the total and LDL cholesterols decreased in those consuming the products for 8 weeks (Quilez and others 2003). A subsequent sensory evaluation indicated that no difference was detected between the products with and without phytosterols at the dose level (Quilez and others 2006).

With regard to bakery production method, the increasing popularity of frozen dough and frozen par-baked bakery products has been driven mainly by the economic advantage of a centralized manufacturing and distribution process as well as the standardization of product quality. These products, bread in particular, do not demand specialized workers and offer the possibility of making "fresh" products available at any time of the day.

The quality of bread made out of frozen dough is influenced by dough formulation, as well as process parameters such as dough mixing time, freezing rate, storage duration, and thawing rate. These factors may act either independently or synergistically to reduce yeast

activity, which results in reduced carbon dioxide production or in damage to the gluten network, which in turn results in poor CO_2 retention and poor baking performance (Lucas and others 2005). The inclusion of improvers in bread formulation, such as emulsifiers and hydrocolloids, may overcome such problems associated with frozen dough. Selomulyo and Zhou (2007) provide a comprehensive review of the effects of freezing storage and dough improvers on the quality of frozen bread dough and the corresponding bread.

On baking equipment, automation and optimal control systems of the bakery making processes are among the major focuses of new development. However, to successfully design an advanced process control system for a bakery making process heavily depends on a good understanding of the process, its responses to the operation conditions and their interactions. This understanding needs to be quantitative. In other words, a good model, particularly a good dynamic model of the process, needs to be established. Bakery making involves complicated transformations from raw ingredients to dough and further to the final product. This makes it very hard to establish a high-quality model. However, there have been persistent efforts and progresses towards modeling the various stages of bakery making using advanced modeling techniques, as described in the previous section.

14.8 SUMMARY

Bread and bakery products are among the popular food items with a long history of development. Varieties of bakery products have been produced with regard to technology development and consumer preference. The main ingredients for bakery products include flour, water, sugar, fat, and salt. Each ingredient imparts a different effect on the quality of the final product. The ingredients should be formulated in accordance with the required rheological properties of dough and batter. The degree of gluten development seems to play the most important role in achieving the desired product characteristics. In addition to formulation, processes including dough making, proving, baking, cooling, and packaging are all among the important steps in delivering a high-quality end product. Different process operating methods and conditions are required to produce different product varieties. To obtain a longer shelf-life, packaging has to be carefully designed. Mathematical modeling could be an effective tool in optimizing both product formation and manufacturing process.

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15

Muffins and Bagels

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15.1 Muffins 280
15.1.1 Background Information 280
15.1.1.1 History of Muffins 280
15.1.1.2 Health Concerns 280
15.1.1.3 Food Labeling and Health Claims 283
15.1.1.4 Food Labeling Standards for Organically Grown Foods 284
15.1.1.5 Ingredient Labeling for Possible Allergens 284
15.1.2 Preparation of Cake-Type Muffins 285
15.1.2.1 Selection and Scaling of Ingredients 285
15.1.2.2 Processing 291
15.1.3 Finished Product 292
15.1.4 Muffin Evaluation 292
15.1.4.1 Volume 292
15.1.4.2 Contour of the Surface 292
15.1.4.3 Color of Crust 292
15.1.4.4 Interior Color 293
15.1.4.5 Cell Uniformity and Size 293
15.1.4.6 Thickness of Cell Walls 293
15.1.4.7 Texture 293
15.1.4.8 Flavor 293
15.1.4.9 Aftertaste 293
15.1.4.10 Aroma 293
15.1.4.11 Mouthfeel 293

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

15.2 Bagels 293
15.2.1 History of Bagels 293
15.2.2 Preparation 295
15.2.2.1 Selection and Scaling of Ingredients 295
15.2.2.2 Processing 296
15.2.3 Bagel Evaluation 299
References 303

15.1 MUFFINS

15.1.1 Background Information

15.1.1.1 History of Muffins. English muffins originating in London were made from yeast dough, in contrast to the quick-bread muffins served in early America. Muffins are described as a quick bread because "quick-acting" chemical leavening agents are used instead of yeast, a "longer acting" biological leavening agent. Muffins have become increasingly popular as a hot bread served with meals or eaten as a snack. Freshly baked muffins are served in restaurants and bakeries, and consumers can buy packaged ready-to-eat muffins from grocery stores and vending machines. With the availability of dry mixes, frozen muffin batter, and predeposited frozen muffins available on the wholesale market, it is possible for restaurants and small bakeries to serve a muffin of a consistently high quality. Muffin mixes that are lower in fat, carbohydrates, and calories are available for home and institutional use for individuals with cardiovascular disease and diabetes.

15.1.1.2 Health Concerns. The economic burden of chronic disease is a worldwide problem. Chronic diseases contributed to 60% of the deaths worldwide in 2001 (WHO 2003a). The increasing rate of obesity and the aging of the population are expected to impact the burden of chronic disease. Those with obesity are at greater risk and have an earlier onset of the chronic diseases of diabetes, cardiovascular disease, cancer, and stroke. Aging increases the risk for all chronic diseases. Nearly one-quarter of the population in developed countries is made up of those over 60 years of age, with expectations for the numbers to increase to one-third of the population by 2025 (WHO 2002a).

The problems of overweight and obesity are growing rapidly around the world and coexist with malnutrition in developing countries (WHO 2003a). Surveys of U.S. adults carried out in 1999–2000 showed that 64% of adults were overweight and 30% were obese (Flegal and others 2002). The percentage of children and adolescents in the United States who are overweight has tripled in the past 30 years, with 15% of 6- to 19-year-olds being overweight in 1999–2000 (Ogden and others 2002). Obesity rates have increased threefold or more in some parts of North America, Eastern Europe, the Middle East, the Pacific Islands, Australasia, and China since 1980 (WHO 2002b). The prevalence rates of overweight and obesity are growing rapidly in children and adults in such countries as Brazil and Mexico, where malnutrition and obesity coexist in the same households (Chopra 2002). Countries with the highest percentage (5–10%) of overweight preschool children are from the Middle East (Qatar), North Africa (Algeria, Egypt, and Morocco), and Latin America and the Caribbean (Argentina, Chile, Bolivia, Peru, Uruguay, Costa Rica, and Jamaica) (de Onis and Blossner 2000).

Globalization of food and the availability of energy-dense snacks and "fast foods" have had a significant impact on dietary patterns and the incidence of chronic disease in both

developing and developed countries (Hawkes 2002). For example, Coca-cola and Pepsi soft drinks and McDonald's, Pizza Hut, and Kentucky Fried Chicken fast foods are now available worldwide (Hawkes 2002). Changes in dietary patterns combined with a sedentary life-style have increased the rates of obesity and chronic disease. Dietary factors related to chronic disease are excessive intakes of calories, fat (especially saturated fat), and sodium, and low intakes of fruits and vegetables and wholegrain breads and cereals (WHO 2004). National dietary guidelines recommend limiting intakes of total fat, saturated fat, trans fat, cholesterol, free sugars, and sodium, and they promote dietary fiber from wholegrain breads and cereals, fruits, and vegetables (WHO 2003b). In 2002, consumers in the United States reported making food choices in an effort to avoid fat, sugar, calories, and sodium, and to increase fiber intake (NMI 2003). Consumers chose fat-free foods or foods low in fat 74–80% of the time and selected low-calorie foods and low-sodium foods 76–67% of the time, respectively. High-fiber foods were chosen 75% of the time, and 40% of consumers reported using organic foods (NMI 2003).

The food industry has responded to concerns of consumers and public health officials by developing "healthy" food products, lower in saturated fat, trans fat, cholesterol, sodium, sugar, and calories. New ingredients have been developed by food scientists in the government and industry to use as fat and sugar replacers in preparing baked products that are lower in calories and saturated and trans fats (Tables 15.1 and 15.2).

TABLE 15.1 Ingredients Used as Fat Replacers in Baked Products.

Brand Name	Composition	Supplier
	Carbohydrate-Based	
Beta-Trim™	Beta-glucan and oat amylodextrin	Rhodia USA, Cranbury, NJ
Fruitrim®	Dried plum and apple puree	Advanced Ingredients, Capitola, CA
Just Like Shorten™	Prune and apple puree	PlumLife division of TreeTop, Selah, WA
LighterBake™	Fruit juice, dextrins	Sunsweet, Yuma City, CA
Oatrim®	Oat maltodextrin	Quaker Oats, Chicago, IL
Paselli FP	Potato maltodextrin	AVEBE America, Inc., Princeton, NJ
Z-Trim	Multiple grain fibers	U.S. Department of Agriculture
	Low- and Noncaloric-Lipid Based	
Enova™	Diglycerides	Archer Daniels Midland/Kao LLC, Decatur, IL
Benefat® Salatrim/ Caprenin	Triglycerides modified by substituting short- or medium-chain fatty acids	Danisco Culter, New Century, KS Proctor and Gamble, Cincinnati, OH
Olestra/Olean®	Sucrose polyester	Proctor and Gamble, Cincinnati, OH

TABLE 15.2 Ingredients Used as Sugar Replacements in Baked Products.

Sweetener	Brand Name	Sweetness Compared to Sucrose	Supplier
Acesulfame-K	Sunett®	200% sweeter	Nutrinova, Somerset, NJ
Sucralose	Splenda®	600% sweeter	Splenda, Inc., Ft. Washington, PA

TABLE 15.3 Ingredients Marketed for Specific Health Benefits.

Brand Name	Composition	Health Benefit	Supplier
Caromax™ Carob fiber	Carob fruit fiber; soluble fiber, tannins, polyphenols, lignan	Lower serum cholesterol	National Starch and Chemical, Bridgewater, NJ
FenuPure™	Fenugreek seed concentrate; galactomannan	Regulate blood glucose; Lower serum cholesterol	Schouten USA, Inc., Minneapolis, MN
Fibrex®	Sugar beet fiber; soluble fiber, lignan	Lower serum cholesterol Regulate blood glucose	Danisco Sugar, Malmo, Sweden
MultOil	Diglycerides &+ phytosterols	Lower serum cholesterol	Enzymotec, Migdal HaEmeq, Israel
Nextra™	Decholesterolized tallow and corn oil; free of trans fat	Reduce the risk for coronary heart disease	Source Food Technology, Durham, NC
Novelose 240	Corn fiber; high amylose, resistant fiber	Reduce risk for colon cancer	National Starch and Chemical, Bridgewater, NJ
Nutrifood®	Fruit and vegetable liquid concentrates; source of antioxidants: carotenoids, anthocyanins, polyphenols	Reduce risk for chronic diseases: cancer, diabetes, and cardiovascular disease	GNT USA, Inc., Tarrytown, NY GNT Germany, Aachen, Germany
OatVantage™	Beta-glucans, a soluble fiber	Lower serum cholesterol	Nurture, Inc., Devon, PA
Tonalin® CLA	Conjugated linoleic acid made from sunflower oil	Weight management	Cognix Corp. USA, Cincinnati, OH
Vegapure® Sterol Esters	Sitosterol, campersterol, and stigmasterol esterified with sunflower oil fatty acids	Lower serum cholesterol	Cognis Corp. USD, Cincinnati, OH

The newest category of ingredients is concentrated bioactive compounds with specific health benefits (Table 15.3) (Pszczola 2002a). These ingredients are added to formulations during food processing to enhance the health benefits of specific food products or to develop "functional foods". Individual foods, such as apples, blueberries, oats, tomatoes, and soybeans, are being marketed as functional foods because of the health benefits of components of these foods. For example, diets that include oat fiber and soy protein lower serum cholesterol, and lycopene in tomatoes reduces the risk of prostate cancer. Apples and blueberries contain unique antioxidants shown to reduce the risk for cancer (Pszczola 2001). Examples of bioactive ingredients available to the baking industry are OatVantage™ (Nature Inc., Devon, Pennsylvania), a concentrated source of soluble fiber, and FenuPure™ (Schouter USA, Minneapolis, Minnesota), a concentrated source of antioxidants from fruits and vegetables.

15.1.1.3 Food Labeling and Health Claims. The Nutrition Labeling and Education Act (NLEA), issued by the Food and Drug Administration (FDA) in the United States in 1990, to be effective in 1994, required food labels to include nutrition content on all packaged foods (FDA/CFSAN 1994). Information required on the Nutrition Facts portion of the food label are the serving size and the amount per serving of calories, protein, fat, saturated fat, cholesterol, carbohydrates, fiber, sodium, calcium, vitamins A and C, and iron. A 1993 amendment to the NLEA authorized food manufacturers to add health claims related to specific food components (Federal Register 2003) (Table 15.4). However, for many "functional foods", the scientific evidence to meet FDA criteria to make health claims is lacking (Wahlqvist and Wattanapenpaiboon 2002). A 2003 amendment to NLEA requires that trans fatty acids be listed under saturated fat on the Food Facts label (DHHS/FDA 2003). The Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations World Health Organization (FAO/WHO) Codex Guidelines on Nutrition Labeling adopted in 1985 are similar to the NLEA implemented by the FDA in 1994 (Hawkes 2004). The Codex Alimentarius Commission adopted the Codex Guidelines for the use of Nutrition Claims on food labels in 1997 (Hawkes 2004). Codex standards are voluntary, and each country within the United Nations is free to adopt food-labeling standards. The European Union, which includes 25 member states in Europe, also sets guidelines for nutrition labeling and nutrition claims, subject to the requirements of the individual member states.

The Food Standards Agency of the United Kingdom was established in 2000 as the regulatory agency to set policy for food labeling in Great Britain and Northern Ireland (FSA 2005). Food Standards Australia and New Zealand (FSANZ 2005) specifies the requirements for food labeling in these countries. Health Canada published new food labeling regulations on January 1, 2003, making nutrition labeling mandatory for most foods and allowing diet-related health claims on food labels for the first time (Health Canada 2005).

TABLE 15.4 Health Claims Approved for Food Labeling in the United States.

Food Component	Health Claim
Calcium	Osteoporosis
Dietary fat	Cancer
Dietary sugar alcohol	Dental caries
Dietary saturated fat and cholesterol	Coronary heart disease
Fiber-containing grain products, fruits, and vegetables	Cancer
Folate	Neural tube defects
Fruits and vegetables	Cancer
Fruits, vegetables, and grain products that contain fiber, particularly soluble fiber	Coronary heart disease
Plant sterols/stanol esters	Coronary heart disease
Potassium	High blood pressure and stroke
Sodium	Hypertension
Soy protein	Coronary heart disease
Wholegrain foods	Heart disease and certain cancers

Source: FDA/CFSAN (1994).

15.1.1.4 Food Labeling Standards for Organically Grown Foods. The Organic Foods Production Act of 1990 passed by the U.S. Congress required the U.S. Department of Agriculture to develop certification standards for organically produced agricultural products (AMS/USDA 2003). Producers who meet the standards may specify the percentage of the product that is organic on the food label if 70% or more of the ingredients in the product are organically grown (AMS/USDA 2003). The Codex Alimentarius Commission has also published standards for labeling organically grown foods (FAO/WHO 2001). Organic fruits and vegetables are produced without using conventional pesticides, petroleum-based fertilizers, or sewage-sludge-based fertilizers. Animal products identified as organic come from animals that are given organic feed but are not given antibiotics or growth hormones. Food products that have been developed through genetic modification cannot be labeled as organically grown foods (FAO/WHO 2001; AMS/USDA 2003).

15.1.1.5 Ingredient Labeling for Possible Allergens. The Codex Alimentarius Commission of FAO/WHO and FDA/CFSAN require that food labels list all ingredients known to cause adverse responses in those with food allergies or sensitivities (FDA/ORA 2001; Hawkes 2004). The FDA requires listing of ingredients from eight foods that account for 90% of all food allergies. These foods are peanuts, soybeans, milk, eggs, fish (e.g., bass, flounder, or cod), crustacean shellfish (e.g., crab, lobster, or shrimp), tree nuts (almonds, pecans, or walnuts), and wheat (FDA/ORA 2001). A proposed rule to define and permit food labels to use the term gluten-free is a part of the Food Allergen Labeling and Consumer Protection Act of 2004 (FDA/CFSAN 2004a). Food manufacturers in the United States are required to list foods from these eight major allergens on the food label (FDA 2005). Codex standards require listing ingredients from these same eight foods plus all cereals that contain gluten (rye, barley, oats, and spelt), lactose, and sulfite in concentrations of 10 mg/kg or more (Hawkes 2004). Gluten, lactose, and sulfite are listed on food labels because these substances cause distress for some consumers, even though these substances are not considered allergens. Individuals with celiac disease or gluten intolerance eliminate all sources of gluten from the diet. A small percentage of individuals lack lactase, the enzyme needed to digest lactose, and avoid dairy products and all other foods with lactose additives.

Food processing plants are required to follow Good Manufacturing Practices (GMPs) to avoid possible cross-contamination with trace amounts of allergens during processing. An example of possible cross-contamination is using the same plant equipment to prepare nut-free muffins after the equipment has been used to prepare muffins with nuts (Taylor and Hefle 2001). An example of GMP is dedicating food processing plants to the production of allergen-free foods (Taylor and Hefle 2001).

Small bakeries (defined by the number of employees or annual gross sales) and restaurants are exempt from the FDA food labeling requirements. Food labeling to identify foods that have been genetically modified through bioengineering (GM) is voluntary (FDA/CFSAN 2001). However, because of consumer concerns about GM foods, managers of bakeries may choose to include a statement on the ingredient label such as, "We do not use ingredients produced by biotechnology", (FDA/CFSAN 2001). Consumers with food allergies have learned to read the lists of ingredients on food labels to identify any possible sources of allergens. Managers of small bakeries that use nuts or soy flour in their operations, but are unable to follow GMPs because of the added cost, may choose to alert consumers with a statement on the ingredient label such as, "This product was made on equipment

TABLE 15.5 Muffin Formulas Listed by Baker's Percent and Weight.

	Basic Cake Muffin (Baker's %)	Weight (g)	Bran Muffin (Baker's %)	Weight (g)
		Ingredients		
Flour	100.00	1000	–	–
Bread flour	–	–	50.0	500
Cake flour	–	–	18.75	187
Bran	–	–	31.25	312
Sugar	60.00	600	31.25	312
Baking powder	5.00	50	1.50	15
Baking soda	–	–	2.20	22
Salt	1.25	12	1.50	15
Milk powder	7.50	75	12.50	125
Molasses	–	–	37.50	375
Shortening	40.00	400	18.75	187
Whole eggs (liquid)	30.00	300	12.50	125
Honey	–	–	19.00	190
Water	60.00	600	100.00	1000
Raisins	–	–	25.00	250
Total	303.75	3037	361.70	3615

Specifications

Mixer: Hobart N-50 with 5-quart bowl and paddle agitator.

Directions for basic cake muffin formula:

- Blend dry ingredients together by mixing for 1 min at low speed
- Add shortening and eggs and mix for 1 min at low speed
- Add water and mix for 1 min at low speed
- Scaling weight: 2 and a 1/2 oz. batter
- Yield: 3 dozen muffins
- Bake at 205°C for 19–21 min in a gas-fired reel oven

Directions for bran muffin formula:

- Blend dry ingredients and mix for 1 min at low speed
- Add shortening, eggs, honey, molasses and 50% (500 g) of the water and mix for 1 min at medium low speed
- Add the remaining water and mix for 1 min at low speed
- Add raisins and mix at low speed for 3 min or until raisins are dispersed
- Scaling weight: 3 oz. batter
- Yield: 3 dozen muffins
- Bake at 193°C for 20–25 min in a gas-fired reel oven.

Sources: Benson (1988), Doerry (1995).

that also makes products containing tree nuts". Making a decision to sell bakery products made with organic ingredients requires assessing the market for these products, the availability of organic ingredients, and the expected income from the operation.

15.1.2 Preparation of Cake-Type Muffins

15.1.2.1 Selection and Scaling of Ingredients. Muffins made by large commercial bakeries are more cakelike, and those made in the home or small institutions tend

to be more breadlike. The differences between cakelike and breadlike muffins are that cakelike muffins are higher in fat and sugar and use soft wheat flours. A common problem encountered in bread-type muffins is tunnel formation resulting from overdevelopment of gluten. However, this problem is avoided in cake-type muffins, because sugar, fat, and soft wheat flours interfere with gluten development and prevent tunnel formation. Bread-type muffins contain 12% of both fat and sugar compared with 18–40% fat and 50–70% sugar in cake-type muffins (Benson 1988).

Formulas for a standard cake-type muffin and a bran muffin are shown in Table 15.5. Ingredient formulas used by commercial bakeries are based on the weight of flour at 100% (Gisslen 2000). The amounts of other ingredients are a percentage of flour weight (Baker's percent). For example,

$$\frac{w_{\mathrm{i}}}{w_{\mathrm{ref}}} \times 100 = \text{baker's } \% \quad \text{(of the specific ingredient)}$$

where w_{i} is the weight of the specific ingredient, and w_{ref} is the total weight of the wheat flour. Therefore, if the weight of another ingredient is the same weight as the flour, the baker's percent for that ingredient is also 100. The advantage of using baker's percent is that batch sizes can be easily increased or decreased by multiplying the baker's percent for each ingredient by the same factor. Weighing all ingredients, including liquids, is faster and more accurate than using volumetric measurements, especially in large commercial bakeries.

Flour. Flour is the primary ingredient in baked products. Flour represents 30–40% of the total batter weight in most cake-type muffins (Benson 1988). Most muffin formulas contain either a blend of cake or pastry flour and a higher protein flour such as bread flour, or all bread flour (Willyard 2000). The protein in flour is needed to provide structure in quick breads made with limited amounts of sugar. Flour contains starch and the proteins glutenin and gliadin, which hold other ingredients together to provide structure to the final baked product. Hydration and heat promote gelatinization of starch, a process that breaks hydrogen bonds, resulting in swelling of the starch granule, which gives the batter a more rigid structure (McWilliams 2001e). Substituting wholewheat flour, wheat germ, rolled oats, or bran for part of the flour is an excellent way to increase fiber. Other types of flour used in muffins include cornmeal, soy, oat, potato, and peanut. An acceptable product is possible when cowpea or peanut flours are substituted for 25% or when wholewheat flour or cornmeal is substituted for 50% of all-purpose flour (Holt and McWatters 1992). Acceptable muffins have been prepared when soy flour was substituted for 10–20% (Sim and Tam 2001) or 100% of all-purpose flour (Bordi and others 2001). None of these flours contains glutenin or gliadin except the wholewheat flour, and large pieces of bran in wholewheat flour cut and weaken gluten strands. Thus, there is minimal gluten development when these flours are used; however, the muffins tend to be crumbly and compact unless other modifications are made in the formula.

Sugar. Amounts of sugar in muffins range from 50 to 70%, based on flour at 100% (Benson 1988). Sugar contributes tenderness, crust color, and moisture retention in addition to a sweet taste. Sucrose promotes tenderness by inhibiting hydration of flour proteins and starch gelatinization. Sugar is hygroscopic (attracts water) and maintains freshness. Corn syrup, molasses, maple sugar, fruit juice concentrates, and honey are used as sweeteners for flavor variety. Honey or molasses is often used as a sweetener in wholewheat or bran muffins to cover the bitter flavor of the bran (Willyard 2000). The quantity of liquid will need to be decreased if these sweeteners are used instead of sucrose because

of the high water content in these syrups. Chemical changes in sugars during baking contribute characteristic flavors and browning. Caramelization of sugar is responsible for the brown crust of muffins. Caramelization involves dehydration and polymerization (condensation) of sucrose (McWilliams 2001c). Reducing sugars such as dextrose, corn syrup, or high-fructose corn syrup are often added to muffins at levels of 1–3% to increase crust color (Willyard 2000). Reducing sugars react with amino acids in flour, milk, and eggs to form a complex that is responsible for the flavor and brown crust of muffins. The reaction between the aldehyde or ketone groups in reducing sugars and the amino acids in protein is described as the Maillard reaction (McWilliams 2001e). The Maillard reaction, together with caramelization, contributes to the characteristic flavor and color of the crust of a baked muffin. Crust temperatures reach 100°C and above, which lowers water activity. Both the high temperature and low water activity are necessary for the Maillard reaction to occur (McWilliams 2001f). Sugar replacers such as acesulfame-K and sucralose (see Table 15.2) can be substituted for all or part of the sugar. These sugar replacers, however, do not contribute to tenderness, browning, or moisture retention; thus, other formula modifications are necessary for an acceptable product. For example, a small amount of molasses or cocoa may be added to substitute for color from the caramelization of sucrose. The shelf-life of muffins prepared without sugar would be very limited.

Fat. Muffins contain 18–40% fat, based on flour at 100% (Benson 1988). Fat contributes to the eating qualities of tenderness, flavor, texture, and a characteristic mouthfeel. Fat keeps the crumb and crust soft and helps retain moisture, and thus contributes to keeping qualities or shelf-life (McWilliams 2001d). Fat enhances the flavor of baked products because flavor components dissolve in fat. Both shortening and vegetable oils are used in muffins.

To meet the demands of the consumer, muffin formulas are being modified to reduce total fat, saturated fat, trans fat, and calories and to increase the amount of monounsaturated and polyunsaturated fat. Canola oil and flaxseed meal are being added to muffins to increase the proportion of monounsaturated fat. Muffins made with reduced fat and polyunsaturated fatty acids (13% safflower oil) were comparable in sensory and physical characteristics to the standard muffin made with shortening at 20% (Berglund and Hertsgaard 1986).

Low-fat and fat-free muffins are available ready to eat and as frozen batters or dry mixes for bake-off. Various fat replacers have been classified by their macronutrient base (see Table 15.1). Carbohydrate- and lipid-based fat replacers can be used to prepare muffins acceptable to the consumer. Lipid-based fat replacers that have the same chemical and physical characteristics as triglycerides are described as fat substitutes (Akoh 1998). These products provide the same characteristics as fat but with fewer calories.

Monoglycerides, diglycerides, and modified triglycerides are examples of fat substitutes that replicate the mouthfeel and sensory qualities of baked products made with shortening. Enova™ (Archer Daniels Midland KAO LLC, Decatur, Illinois) is an example of a diglyceride that is lower in calories than other oils and is being marketed as beneficial in weight management (Pszczola 2003). Benefat® (Danisco Culter, New Century, Kansas) and Caprenin (Proctor and Gamble, Cincinnati, Ohio) are examples of triglycerides modified by substituting shorter-chain fatty acids (Akoh 1998). Sucrose polyesters of six to eight fatty acids are marketed as Olean® (Procter and Gamble, Cincinnati, Ohio), a fat substitute with the same physical qualities as shortening but without the calories, because sucrose polyesters are not digested or absorbed in the human intestinal tract. A commercial shortening product (Nextra™) (Source Food Technology, Durham, North Carolina) made from decholesterolized tallow and corn oil is being marketed to the baking industry as a trans-free fat to replace shortening (Pszczola 2002b). Other methods used by the food

industry to decrease the amount of trans fat are (1) blending hydrogenated fat high in stearic acid with unhydrogenated oils, and (2) interesterfying (rearranging) unhydrogenated oils with saturated-fat-based oils (Hunter 2002).

Carbohydrate-based fat replacers are described as fat mimetics; for example, cellulose, corn syrup, dextrins, fiber, gum, maltodextrins, polydextrose, starches, and fruit-based purees. Z-trim, developed by a U.S. Department of Agriculture Scientist, is a mixture of plant fibers (Inglett 1997). Fat mimetics replicate the mouthfeel and texture of fat in baked products and extend the shelf-life by binding water and trapping air (American Dietetic Association 2005). Acceptable low-fat cake muffins (5% fat) use 2% pregelatinized dull waxy starch and corn syrup (3.6%) to replace fat (Hippleheuser and others 1995).

Fruit purees or pastes of one or more fruits – apples, dates, figs, grapes, plums, prunes, and raisins – are being promoted as fat replacers. Just Like Shorten™ is a mixture of dried prunes and apples. The fruit purees have humectant properties, promote tenderness and moistness, increase shelf-life, and can replace some of the sugar and/or fat in muffins and cakes. Formulas will need to be developed based on adjustments in ingredients when fat replacers are substituted for all or part of the fat in the formula. New formulas need to be prepared and the muffins evaluated using the muffin scorecard (Table 15.6),

TABLE 15.6 Scorecard for Muffins.

Evaluator:
Product:
Date:

Score

External Qualities

1a. Volume Specific Volume: $\pi(r^2) \times$ height $\div$ weight in grams (cm) 1 = low volume, compact cells; 5 = light with moderate cells; 9 = large volume, large cells and/or tunnels

1b. Contour of the surface 1 = absolutely flat; 3 = somewhat rounded; 5 = pleasingly rounded; 7 = somewhat pointed; 9 = very pointed

1c. Crust color 1 = much too pale; 3 = somewhat pale; 5 = pleasingly golden brown; 7 = somewhat too brown; 9 = much too brown

Internal Qualities

1d. Interior color 1 = much too white; 3 = somewhat white; 5 = pleasingly creamy; 7 = somewhat too yellow; 9 = much too yellow

1e. Cell uniformity and size 1 = much too small; 3 = somewhat thick; 5 = moderate; 7 = somewhat too large; 9 = numerous large tunnels

1f. Thickness of cell walls 1 = extremely thick; 3 = somewhat thick; 5 = normal thickness; 7 = somewhat too thin; 9 = much too thin

1g. Texture 1 = extremely crumbly; 3 = somewhat crumbly; 5 = easily broken, 7 = slightly crumbly; 9 = tough, little tendency to crumble

1h. Flavor 1 = absolutely not sweet enough; 3 = not nearly sweet enough; 5 = pleasingly sweet; 7 = somewhat too sweet; 9 = much too sweet

1i. Aftertaste 1 = extremely distinct; 3 = somewhat distinct; 5 = none

1j. Aroma 1 = lack of aroma; 5 = sweet and fresh aroma; 9 = sharp, bitter or foreign aroma

1k. Mouthfeel 1 = gummy, cohesive; 3 = somewhat gummy; 5 = tender, light and moist; 7 = somewhat dry and tough; 9 = tough and hard to chew

Overall Acceptability

1 = very unacceptable; 3 = somewhat acceptable; 5 = very acceptable

Source: McWilliams 2001a.

and the shelf-life also needs to be evaluated. Several formula adjustments may be necessary before an acceptable muffin is developed.

Leavening Agents. The amount of baking powder used in muffins varies between 2 and 6%, based on flour at 100%, with lower amounts in muffins with ingredients that increase acid (Benson 1988). Gases released by a leavening agent influence volume and cell structure. During baking, heat increases gas volume and pressure to expand cell size until proteins are coagulated (McWilliams 2001c). Stretching of the cell walls during baking improves texture and promotes tenderness (McWilliams 2001c). The quantity of leavening used in a baked product depends on the choice of leavening agent as well as other ingredients. Formulation of baking powders considers the amount of leavening acids needed to neutralize baking soda or sodium bicarbonate, an alkaline salt. Double-acting baking powder (most commonly used in muffins) contains both slow- and fast-acting acids (McWilliams 2001e). Fast-acting acids are readily soluble at room temperature, but slow-acting acids are less soluble and require heat over an extended period of time to release carbon dioxide. Formulations of slow- and fast-acting acid leavening agents control the reaction time and optimize volume (Borowski 2000). An example of a formulation to neutralize sodium bicarbonate is a mixture of slow- and fast-acting acids – monocalcium phosphate monohydrate (a fast-acting acid) combined with sodium aluminum sulfate (a slow-acting acid). Development of baking powder requires consideration of the unique neutralizing value (NV) and the rate of reaction (ROR), or the percent of carbon dioxide released during the reaction of sodium bicarbonate with a leavening acid during the first 8 min of baking (Borowski 2000).

Baking soda is used in addition to double-acting baking powder when muffins contain acidic ingredients such as sour cream, yogurt, buttermilk, light sour cream, molasses, and some fruits and fruit juices (McWilliams 2001e). Baking soda in the amount of 2–3% is added to acidic batters (Benson 1988).

Sodium carbonate is a product of an incomplete reaction in formulas with excess sodium bicarbonate. Excess sodium carbonate results in a muffin with a soapy, bitter flavor and a yellow color because of the effect of an alkaline medium on the anthoxanthin pigments of flour (McWilliams 2001f). Also, formulas with too much baking powder or soda result in a muffin with a coarse texture and low volume because of an overexpansion of gas, which causes the cell structure to weaken and collapse during baking. Inadequate amounts of baking powder will result in a compact muffin with low volume. Figures 15.1 and 15.2 show different chemical reactions for fast-acting and slow-acting baking powders (McWilliams 2001e).

Whole Eggs. Liquid egg contributions to muffin batter are 10–30%, based on flour at 100%, and dried egg powder contributions are 5–10% (Benson 1988). Eggs provide flavor, color, and a source of liquid. Upon baking, the protein in egg white coagulates to provide structure. Adding egg whites to muffin batter provides structure to the finished product and produces a muffin that is easily broken without excessive crumbling (Stauffer 2002). Substituting egg whites for whole eggs, however, will result in a dry, tough muffin

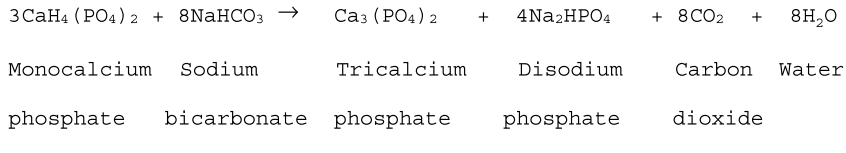

Figure 15.1 *Formation of bicarbonate of soda from a fast-acting acid salt.*

Step 1.

$$Na_2(Al)_2(SO_4)_4 + 6H_2O \rightarrow 2Al(OH)_3 + Na_2SO_4 + 3H_2SO_4$$

Sodium aluminum sulfate — Water — Aluminum hydroxide — Sodium sulfate — Sulfuric acid

Step 2.

$$3H_2SO_4 + 6NaHCO_3 \rightarrow 3Na_2SO_4 + 6H_2CO_3$$

$$6H_2CO_3 \downarrow \rightarrow 6CO_2 + 6H_2O$$

Sulfuric acid — Sodium bicarbonate — Sodium sulfate — Carbonic acid — Carbon dioxide — Water

Figure 15.2 *Formation of bicarbonate of soda and carbon dioxide from a slow-acting acid salt.*

unless the formula is adjusted to increase the amount of fat (Stauffer 2002). Fat in the yolk acts as an emulsifier and contributes to mouth-feel and keeping qualities.

Nonfat Dry Milk Powder. Milk powder contributions to muffin batter are 5–12%, based on flour at 100% (Benson 1988). Milk powder is added to dry ingredients, and water or fruit juice is used for liquid in muffin formulas. Milk powder binds flour protein to provide strength, body, and resilience – qualities that are helpful in reducing damage during packing and shipping (Willyard 2000). In addition, milk powder adds flavor and retains moisture. The aldehyde group in lactose in milk combines with the amino group in protein upon heating, contributing to Maillard browning.

Sodium Chloride. The amount of salt in muffins is 1.5–2%, based on flour at 100% (Benson 1988). The function of sodium chloride is to enhance the flavor of other ingredients. Sodium chloride may be omitted from the formula without compromising flavor, if other ingredients such as dried fruit or spices are added for flavor.

Liquids. Liquids perform several functions in baked products (Benson 1988). These include dissolving dry ingredients, gelatinization of starch, and providing moistness in the final baked product. Insufficient liquid results in incomplete gelatinization of the starch and a muffin without the structure to support expansion of air volume. The muffins will have nonuniform cell structure, overly crumbly texture, low volume, and a dip or "M" fault in the top.

Additional Ingredients. Other ingredients are often added to muffins to provide variety in flavor, texture, and color and to increase specific nutrients or health components such as fiber, vitamins and minerals, or antioxidants from fruit and vegetable extracts. Part of the flour may be replaced with cornmeal, bran, or wholewheat, oat, or other flours to increase the fiber content. Adjustments in the amount of water in the formula are necessary when wholewheat flour, bran, or other concentrated sources of fiber are added, because fiber absorbs a great deal of water (Willyard 2000). An example of a concentrated

source of fiber is Caromax™ (National Starch and Chemical, Bridgewater, New Jersey) (Pszczola 2001). Nutrifood® (GNT USA, Tarrytown, New York), a liquid concentrate marketed as a blend of the antioxidants carotenoids, anthocyanins, and polyphenols, is an example of a bioactive ingredient (Pszczola 2002a).

Other ingredients can be substituted for part of the liquid; for example, applesauce, bananas, shredded carrots, or zucchini. Variations in texture are achieved by adding fresh fruits such as apples or blueberries, or dried fruits such as dates, raisins, or apricots. Nuts and poppy seeds complement the flavor of sweet muffins, and grated cheese, whole-kernel corn, green peppers, chopped ham, and bacon add interest to corn muffins. Added flavorings include cinnamon, nutmeg, allspice, cloves, and orange or lemon zest. Topping mixtures such as chopped nuts, cinnamon, and sugar are added to the batter after depositing.

15.1.2.2 Processing

Stage 1 — Mixing. There are two primary methods for mixing muffins – the cake method and the muffin method. The cake method involves creaming sugar and shortening together, followed by addition of liquid ingredients and the final addition of dry ingredients. The muffin method of mixing involves two to three steps. First, dry ingredients are mixed together; second, shortening or oil and other liquids are mixed together; and third, the liquids are added to the dry ingredients and mixed until the dry ingredients are moistened. Additional ingredients are added at the end of the mixing cycle or after depositing the muffin batter. Institutional or commercial bakeries use a mixer on slow speed for 3–5 min. Inadequate mixing results in a muffin with a low volume, as some of the baking powder will be too dry to react completely.

Stage 2 — Depositing. The traditional size of muffins is 2 ounces, although today muffins are marketed in a wide range of sizes from 1/2-ounce mini muffins to muffins 5 ounces or larger in size (Willyard 2000). For institutions or bakeries, small batter depositors are available that will deposit four muffins at a time. Also available are large piston-type depositors that maintain accurate flow of the batter (Benson 1988).

Stage 3 — Baking. Many physical and chemical changes occur in the presence of heat to transform a liquid batter into a final baked muffin. Solubilization and activation of the leavening agent generate carbon dioxide, which expands to increase the volume of the muffin. Gelatinization of starch and coagulation of proteins provide permanent cell structure and crumb development. Caramelization of sugars and Maillard browning of proteins and reducing sugars promote browning of the crust. Reduced water activity facilitates Maillard browning as well as crust hardening (McWilliams 2001f). The choice of oven, baking pans, and baking temperature influences the final baked product (Benson 1988). A good flow of heat onto the bottom of the pan is necessary to produce a good product. Muffin tins are usually placed directly on the shelf or baking surface. The appropriate oven temperature is related to scaling and the type of oven. Standard 2-ounce muffins are baked at 204°C or slightly higher in a deck oven.

Deck ovens may be stacked and are often used in small retail bakeries, because these are less expensive and easier to maintain than reel or rotary ovens. Reel ovens consist of an insulated cubic compartment 6 or 7 feet high. A Ferris wheel-type mechanism inside the chamber moves four to eight shelves in a circle, allowing each shelf to be brought to the door for adding or removing muffin tins from the shelves (Matz 1988). Retail bakers often

prefer the reel oven as several hundred to several thousand pounds of batter can be baked each day. Rack ovens may be stationary, or the racks may be rotated during baking.

Stage 4 — Cooling. Products should be cooled prior to wrapping. This allows the structure to "set" and reduces the formation of moisture condensation within the package. Condensed moisture creates an undesirable medium that promotes yeast, mold, and bacterial growth and spoilage.

Stage 5 — Packaging. Muffins may be wrapped individually, in the tray in which they are baked, or transferred into plastic form trays for merchandizing (Benson 1988). The shelf-life of muffins is 3–5 days for wrapped muffins, and 4–7 days for those packaged in foil or plastic wrap. The storage life of muffins is significantly influenced by exposure to oxygen and moisture (Rice 2002). Cake-type muffins have a longer shelf-life than bread-type muffins because of their high sugar content and lower water activity (Willyard 2000). Added ingredients such as cheese, ham, and dried fruits, which are high in sodium or sugar content, reduce water activity and may increase shelf-life.

15.1.3 Finished Product

A muffin fresh out of the oven will vary in appearance based on the formula (whether the formula is for a cake- or bread-type muffin), the size of the muffin (mini muffin or mega muffin), and the desired shape, from flat or mushroom-shaped tops to the traditional bell-shaped muffin (Willyard 2000). In general, a desirable muffin product has a symmetrical shape, a rounded top that is golden brown in color, cells that are uniform and moderate in size, and a sweet flavor and pleasant aroma. It should be tender and moist, be easily broken apart, and be easy to chew with a pleasant aftertaste.

15.1.4 Muffin Evaluation

During the process of developing or modifying muffin formulas, bakers can use Table 15.6, Scorecard for Muffins, to evaluate muffins. Large commercial bakeries may use sophisticated methods to evaluate bakery products, for example, gas chromatography to evaluate flavor components.

15.1.4.1 Volume. Compact muffins with small cells or large muffins with peaked tops and tunnels are undesirable in all types of muffins. Diameter is a more important criterion than volume for evaluating mushroom and flat-topped muffins. For bell-shaped muffins, volume can be evaluated objectively by measuring the height and width of the muffin and calculating the volume ($\pi[r^2] \times$ height). To determine the height and width, measure the height of the muffin at the highest point, then slice off the top of the muffin and measure the diameter of the muffin. The volume also can be directly measured by Rapeseed displacement using a special volume meter (National Mfg., Lincoln, Nebraska).

15.1.4.2 Contour of the Surface. The muffin should be rounded and golden brown in color with a pebbled surface.

15.1.4.3 Color of Crust. Crust color should be a pleasing golden brown, not pale or burnt.

15.1.4.4 Interior Color. Crumb color should be a pleasant creamy color, not white and not too yellow. Crumb color will be darker with wholegrain flour or when added ingredients such as nuts, dried fruits, or spices are used.

15.1.4.5 Cell Uniformity and Size. Cell structure can be evaluated by making a vertical cut in the muffin to form two equal halves and then making an ink print or photo copy (McWilliams 2001b). A desirable muffin should have a uniform cell structure without tunnels.

15.1.4.6 Thickness of Cell Walls. Uniform thick-walled cells are desirable. Coarseness, thin cell walls, uneven cell size, and tunnels indicate poor grain.

15.1.4.7 Texture. Texture depends on the physical condition of the crumb and is influenced by the grain. A desirable muffin should be easily broken and slightly crumbly. Extreme crumbling, and toughness with lack of crumbling, are undesirable characteristics.

15.1.4.8 Flavor. An acceptable muffin should have a pleasingly sweet flavor. Flat, foreign, salty, soda, sour, or bitter tastes are undesirable.

15.1.4.9 Aftertaste. An acceptable muffin should have a pleasant, sweet aftertaste, not bitter or foreign.

15.1.4.10 Aroma. Aroma is recognized by the sense of smell. The aroma may be sweet, rich, musty, or flat. The ideal aroma should be pleasant, fresh, sweet, and natural. Sharp, bitter, or foreign aromas are undesirable.

15.1.4.11 Mouthfeel. Mouthfeel refers to the textural qualities perceived in the mouth. Characteristics can be described as gritty, hard, tough, tender, light, or moist. A desirable muffin is tender, light, and moist and requires minimal chewing.

15.2 BAGELS

15.2.1 History of Bagels

Bagels originated in southern Germany in the 1600s to honor the successful campaign of the Polish King Jan Cobleskill and his Christian horsemen against the Turkish invasion of Vienna, Austria. Dough used to make hard rolls was formed into the shape of a stirrup by pulling the dough around to form a half circle. The word bagel was derived from the Yiddish and German word for a round loaf of bread, bugel, and from the German word for stirrup, buegel (Meloan and Doerry 1988).

Bagels were developed by Jewish bakers in Eastern Europe and were sold by street vendors in small towns in Poland. Because of the circular shape, bagels symbolized the eternal circle of life and a protection against evil spirits (Roden 1996). Bagels followed Jewish immigration in the early 1900s from Eastern to Western Europe, Canada, and the United States.

TABLE 15.7 Bagel Formulas Listed by Baker's Percent and Weight.

	Basic Bagel Formula (Baker's %)	Weight (g)	Oregon Hazelnut Bagel Formula (Baker's %)	Weight (g)
	Ingredients			
Bread flour (11% protein)	95.2	2856	–	–
Vital wheat gluten	4.8	144	–	–
High-gluten wheat flour	–	–	100	4540
Sugar	3	90	–	–
Salt	2	60	2.0	80
Vegetable shortening	3	90	–	–
Vegetable oil	–	–	2.0	136
Compressed yeast	2	60	2.25	102
Water	50	1500	48.0	2180
Oregon hazelnuts (sliced thin)	–	–	25	1135
Total	160.0	4800	183.25	8173

Specifications

Mixer: Hobart A-200 with a dough arm and 20-quart bowl

Directions for basic bagel formula:

- Add all ingredients to the mixing bowl and mix at first speed for 2 to 3 min or until the dough comes together
- Mix at second spend for 8 to 10 min or until the gluten in the dough is developed
- Scale dough into 2.35 oz. (70 g) pieces and roll into cylinders 6 in. long
- Slightly moisten and join the cylinder ends to form a circle
- Roll the dough to seal the ends together
- Place the formed dough onto a sheet pan dusted with corn meal or lined with silicone paper
- Retard the dough by storing for approximately 18 h at 4°C
- Proof for 20 min at 30°C
- Place each piece of dough into water heated to 93°C and float the bagel on the water surface for 1 min, turn the bagel over and cook for another minute
- Remove bagels from the water with a wire sieve and place on a screen to dry for about 5 min
- Bake bagels on oiled sheet pans or aluminum sheet pans lined with silicone treated paper
- Scaling weight: 2.35 oz. (70 g) batter
- Yield: 5 dozen bagels
- Bake at 232°C for 17 min

Directions for Oregon Hazelnut Bagel formula:

- Maintain temperature of ingredients during preparation and proofing between 25°C and 27°C
- Add all ingredients to the mixing bowl and mix at first speed for 2 to 3 min or until the dough comes together
- Mix at second speed for 8 to 10 min or until the gluten in the dough is developed
- Proof for 10 min
- Scale at 2 oz. (60 g) with an automated bagel forming machine and place on silicone lined sheet pans
- Proof for 30 min
- Bake in a low-pressure steam oven; turn the bagels after the first 5 min of baking to produce a rounded surface on both sides of the bagel
- Scaling weight: 2 oz. (60 g) batter
- Yield: 12 dozen bagels
- Bake at 198°C for 17 min

Sources: Meloan and Doerry 1988; Hazelnut Marketing Board, http://oregonhazelnut.org/formula.htm, August 2004.

Long known as a Jewish ethnic food available only in large metropolitan centers, bagels have been available since the 1970s in bakeries (including franchise bagel bakeries), restaurants, and grocery stores across the United States and Canada. Retail bakers can purchase bagels in several forms – ready to eat, baked and frozen, frozen dough that has been shaped, proofed and boiled in water, or frozen dough (Petrofsky 1986). The bagel is a traditional breakfast bread served with cream cheese; however, bagel bakeries also serve bagel sandwiches. Bagels come in a variety of sizes, shapes, and flavors with added ingredients such as cheese, fruit, seeds, nut meats, and chocolate chips. In 2004, the number of bagels sold in U.S. grocery stores totaled 223,000,000, representing $507,000,000 in sales (Anon. 2004).

New York style bagels use traditional formulas and preparation methods brought from Eastern Europe. The formula consists of five ingredients: high protein wheat flour, fresh yeast, malt extract, water, and salt. Strips of dough are formed into bagels, allowed to proof, dropped into boiling water and placed on wooden slabs, and put into the oven for drying. The bagels are then turned onto the hearth of a stone oven to finish baking. The product is a bagel with a shiny, hard outer crust, deep amber in color, with a dense, chewy texture inside. Many commercial bakeries modify the formula with the addition of sugar and oil for a softer crumb and longer shelf-life. Egg-bagel formulas made with whole eggs (4–12%, on a flour weight basis) or yolks (1.5–3.0%) provide variety in crumb color and flavor.

Bagel production in large commercial bakeries is often completely automated and does not require human labor. After mixing, the dough is put into the hopper of a dough divider, which scales dough into uniform portions that are delivered to a molder for shaping. Conveyors move shaped bagels into a proofing chamber and then into an automatic cooker, dryer, and tunnel oven. Automatic slicers and packaging systems complete the process (Petrofsky 1986).

15.2.2 Preparation

15.2.2.1 Selection and Scaling of Ingredients.

Flour. Flour represents 56–63% of the total weight of the bagel formula (Table 15.7). High-protein (13.5–14%) flour made from hard wheat is preferred (Petrofsky 1986). An alternative to using high-gluten/high-protein flour is to combine bread flour (11.2% protein) with vital wheat gluten (70% protein) to increase the protein content up to 14%. Bagels require high-gluten flour for a product with the desirable high volume, toughness, and round bottom crust. The gluten proteins, gliadin and glutenin, provide structural support in yeast-leavened bakery products. The high percentage of flour in the formula also contributes to the bagel's flavor.

Sweeteners. The level of sweeteners in bagel formulas is low (0–4%, on a flour weight basis) (Petrofsky 1986). Granulated sugar, malt syrup, honey, molasses, dextrose, corn syrup, or high-fructose corn syrup can be used in bagel formulas. Sweeteners are a source of carbohydrate for yeast fermentation and contribute to crust browning. Formulas with 3% or more sweeteners promote excessive yeast fermentation and overexpansion of dough, which may collapse after boiling, resulting in a bagel with low volume and an overly dark crust. Bagels made with less than 2% sweetener have pale crusts. New York style bagels lack sweeteners and depend on malt extract for crust browning.

Sodium Chloride. Salt levels in bagel formulas range between 1.5 and 2.2%. The functions of salt are to toughen the gluten during mixing and proofing, and to control yeast fermentation after the bagels have been boiled. Bagels lack flavor when salt is omitted from the formula because of the lack of both salt and sugar, as all of the sugar is used for yeast fermentation in the absence of salt. In addition, bagels made without salt have a tendency to over proof and then collapse after removal from the boiling water. Excessive salt results in bagels with a salty taste and a small compact form (due to the retarding effect of salt on fermentation) (Meloan and Doerry 1988).

Fat. Vegetable oil or shortening lubricates the bagel dough and contributes to crumb tenderness. The amount of fat in bagel formulas ranges from 0 to 5%. Bagels made from formulas with 3% fat have a higher volume and are more tender than formulas without fat. Increasing the fat content to 4% results in bagels with surface blisters (Meloan and Doerry 1988).

Yeast. The functions of yeast are dough fermentation and expansion during proofing, boiling, and baking. Compressed yeast is used at a level between 0.5 and 2%. Active or instant dry yeast can be substituted for compressed yeast but requires hydration prior to being added to the formula, as bagel dough has low water absorption. Bagels with 3% yeast over proof and collapse after boiling, resulting in a flat bagel (Meloan and Doerry 1988).

Liquid. The source of liquid in bagels is water at 50–53% on a flour weight basis. Water is necessary for moistening dry ingredients and gluten development. Stiff dough is desirable for holding shape during proofing, boiling, and baking. Formulas with more than 50% water form a soft dough and yield a bagel with low volume and surface blisters.

Additional Ingredients. Bagel variety is achieved by adding additional ingredients during processing. The most common added ingredients are fresh or dried fruit, nutmeats, minced garlic or onions, sesame seeds, poppy seeds, cornmeal, grated cheese, chocolate pieces, and spices. Spices are mixed with the dry ingredients before water is added, and seeds, cornmeal, and other toppings are sprinkled over the bagels just before baking.

15.2.2.2 Processing

Stage 1 — Mixing. The purpose of mixing is to moisten and disperse dry ingredients and develop gluten. Bagel dough is very stiff because of low water absorption, and mixing can overload mixing equipment. Dough quantity should be limited to 60% of mixer capacity to avoid overstressing the mixer (Petrofsky 1986). The mixer is set at the first (lowest) speed to disperse ingredients (approximately 2–3 min); then the mixer is set at the second (higher) speed for 8–10 min for gluten development. An alternative is to mix at a low speed for about 16 min for the total process (Petrofsky 1986). Undermixing stiff bagel dough is more likely to be a problem than overmixing. Undermixing the dough results in poor quality bagels – small and asymmetrical in shape with a blotched crust and a very dense crumb that gives a gummy mouthfeel when eaten (Meloan and Doerry 1988).

Maintaining the dough temperature at 24–27°C during mixing will produce a bagel of high quality. Bagels made at low dough temperatures (<19°C) have a dense crumb and

poor texture, and cracks appear in the crust of bagels made at high dough temperatures (>33°C) (Meloan and Doerry 1988). Some mixers have cooling devices to regulate dough temperature, because heat is generated during mixing. Iced water is also used to regulate dough temperature.

Stage 2 — Scaling and Makeup. Bagels range in size from small cocktail bagels to the very large "super bagel", or "bull bagel". However, most bagels are made from about 2.5–3 ounces (70–90 g) of dough. Bagels can be shaped either manually or with fully automatic bagel-forming machines at a rate of up to 18,000 bagels per hour. Shaped bagels are placed on sheet pans either dusted with cornmeal or lined with silicone-treated paper sheets to prevent the dough from sticking to the pan.

Shaped bagels are typically refrigerated for 12–18 h at 2–6°C to retard yeast fermentation and to allow dough fermentation by lactic acid bacteria. Retardation can be as long as 2 days or can be shortened by increasing the amount of yeast or adding dough conditioners (Petrofsky 1986). A longer fermentation period allows for development of a desirable flavor and aroma but can result in crust blisters that may be undesirable for some consumers (Meloan and Doerry 1988).

Stage 3 — Proofing. Shaped bagels that have been refrigerated to retard fermentation are proofed to allow warming and expansion of the dough before boiling in water. When bagel dough is retarded, yeast fermentation is limited to the relatively short period during proofing, boiling, and transferring to the oven for baking. Bagels are proofed under controlled conditions at 30–33°C and 65–75% relative humidity for 20–30 min to prevent crust formation during proofing (Meloan and Doerry 1988).

Stage 4 — Boiling. Boiling or "kettling" the shaped dough after proofing is unique to bagel production. Boiling sets the protein structure to form a hard crust and gelatinizes the starch to produce a glossy crust. The addition of 2.5% sugar or malt to the boiling water promotes a shiny crust. Omitting the boiling step in bagel production will cause the dough to expand excessively during baking, resulting in a collapsed or flat bagel.

Some large industrial bakeries omit the boiling step, and instead the bagels are baked in an atmosphere of wet steam (Meloan and Doerry 1988). In small-scale production, raw bagels are dropped into a kettle of water at a temperature just below boiling until floating bagels cover the water surface. The bagels will first sink to the bottom of the kettle for about 30 s and then float to the surface. The bagels should be cooked for about 30 s on one side, and then turned and cooked on the other side for another 30 s. The bagels are then removed from the water and placed on a pan. A sieve or screen is an efficient tool for turning and transferring bagels (Petrofsky 1986).

Stage 5 — Baking. Traditionally, bagels were baked on redwood boards placed on oven shelves; the bagels were turned over after 5 min of baking to produce a bagel with a round bottom. Transite or metal plates can be used instead of wood for baking bagels directly on the oven shelf. Bagels can also be baked on oiled or glazed sheet pans or sheet pans lined with parchment paper. Bagels baked on solid pans will have slightly flatter bottoms than bagels baked directly on oven shelves (Petrofsky 1986). However, bagels baked directly on perforated screen pans will have a more symmetrical rounded bottom. A relatively high temperature is needed for baking bagels to develop a good crust color, because

ingredients that promote browning are in low amounts or completely lacking in bagel formulas for example, sugar or other sweeteners, milk, eggs, whey, or soy protein (Meloan and Doerry 1988). Toppings such as caraway seed, poppy seed, sesame seed, minced onion or garlic, cornmeal, or grated cheese are applied to the bagel tops immediately before baking. Bagels are baked at an oven temperature of 204°C for 20–25 min or at 232°C for 17 min, or until golden brown.

Stage 6 — Packaging. Bagels are allowed to cool at room temperature before packaging. Bagels are packed in paper bags if purchased while still warm to allow cooling without condensation. Completely cooled bagels are packed tightly in plastic bags for sale. Bagels have a short shelf-life because of the low fat and sugar content and should be frozen if anticipated storage time is longer than 24 h.

TABLE 15.8 Scorecard for Bagels.

Evaluator:
Product:
Date:

Score

External Qualities

1a. Volume
Specific Volume: $\pi(r^2) \times$ height $\div$ weight in grams (cm) 1 = low volume, shriveled appearance; 5 = light with moderate cells; 9 = large with collapsed top

1b. Contour of the surface
1 = absolutely flat bottom; 5 = pleasingly rounded top and bottom; 9 = somewhat rounded bottom;

1c. Crust surface
1 = irregular, mottled surface; 3 = dull surface; 5 = shiny, smooth surface; 7 = surfac blisters; 9 = surface cracks and/or tears

1d. Crust color
1 = much too pale; 3 = somewhat pale; 5 = pleasingly golden brown; 7 = somewhat too brown; 9 = much too brown, burned appearance

Internal Qualities

1e. Interior color
1 = much too white; 5 = pleasingly creamy; 9 = much too dark

1f. Texture
1 = extremely dense, compact cells; 5 = even cells throughout; 9 = very large cells

1g. Flavor
1 = strong yeast flavor; 3 = bland flavor; 5 = pleasingly sweet; 9 = overly salty flavor

1h. Aftertaste
1 = extremely distinct; 3 = somewhat distinct; 5 = none

1i. Aroma
1 = lack of aroma; 5 = sweet and fresh aroma; 9 = sharp, bitter or foreign aroma

1j. Mouthfeel
1 = gummy, cohesive interior; 3 = somewhat gummy interior; 5 = tender, moist and easy to chew; 7 = somewhat dry and tough; 9 = tough and hard to chew

Overall Acceptability

1 = very unacceptable; 3 = somewhat acceptable; 5 = very acceptable

Source: Adapted from McWilliams 2001a.

TABLE 15.9 Bagel Troubleshooting.

Problem	Possible Cause	Solution
Dull crust	Boiling water too cool	Increase water temperature to 93–100°C
	Oven too cool	Increase oven temperature to 204°C
Bagel is small and hard	Insufficient water in the dough	Adjust water level
	Yeast level too low	Adjust yeast level
	Retardation time not long enough before boiling	Adjust holding time
Bagel is very large and soft	Too much yeast	Adjust yeast level
	Bagels are overproofed	Adjust proof time before boiling
Bagels collapse or shrink during baking	Undermixing of dough	Mix to ensure gluten development
	Bagels are overproofed	Adjust proof time before boiling
Bagels have surface; tears	Temperature during mixing or retardation too high	Adjust temperature during mixing to 24–27°C and during retardation to 30–33°C
Bagel surface; has blisters	Bagels are retarded too long	Adjust proof time
	Too low humidity during proofing	Adjust humidity to 65–75%
Bagel has a flat bottom	Dough is too soft	Mix dough sufficiently to develop gluten
	Flour too weak	Use high-gluten flour or adjust protein to 14% by adding Vital Wheat Gluten

Source: Adapted from Petrofsky 1986.

15.2.3 Bagel Evaluation

A desirable bagel has a round top and bottom with a definite hole in the center; a crust that is hard, shiny, and amber in color; and a tender crumb that is easy to chew. Factors that can contribute to a bagel of poor quality include the quality of ingredients, the formula, and the entire bagel process from mixing, retardation, boiling, and proofing to baking. Refer to Table 15.8, Scorecard for Bagels, and Table 15.9, Bagel Troubleshooting, for use in evaluating bagels.

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GLOSSARY

Allergen: A substance that causes an abnormal immune response in individuals with an allergy to that substance. The most common food allergens are peanuts, milk, eggs, wheat, soy, tree nuts, fish, and shellfish.

AMS/USDA: Agricultural Marketing Service/U.S. Department of Agriculture.

Anthoxanthin: A naturally occurring color pigment in plants and wheat flour. The pigment turns yellow in the presence of an alkaline medium; for example, the crumb is yellow when excessive amounts of baking soda have been added to the muffin batter.

Antioxidant: Naturally occurring compounds, found in plant foods, that provide possible health benefits by quenching free radicals and thus preventing cancer, cardiovascular disease, and other chronic diseases. Color pigments in fruits and vegetables are important sources of antioxidants, for example, proanthocyanidin in blueberries, lycopene in tomatoes, and lutein in spinach.

Baker's percent: Term used by the baking industry to describe the amount of each ingredient by weight for a "recipe" or formula compared with the weight of flour at 100%. Also described as flour weight basis.

Caramelization: Chemical changes in sucrose (dehydration and polymerization) in response to heat during baking; caramelization provides the characteristic color and flavor in baked products.

Carbon dioxide: Gas produced by chemical leavening agents that expands muffin batter during baking.

Cell structure: An internal characteristic of baked products. A desirable cell structure is uniform with moderately sized cells. Factors that influence cell structure are the formula, mixing process, and baking temperature.

Chemical leavening agent: Agents made of a mixture of alkaline bicarbonates and a leavening acid phosphate that is activated by water and baking temperatures to generate carbon dioxide, which expands the muffin batter during baking.

Coagulation: Changes in the structure of protein in flour, milk, and eggs during baking that binds the ingredients; denaturation of protein breaks weak chemical bonds and allows formation of stronger bonds among strands of protein, causing "clumping" and setting the structure of baked products.

Crumb: An internal characteristic of baked products that describes the texture. It is related to tenderness or ease in breaking into pieces, from very crumbly to tough with little tendency to crumble.

Deck oven: A type of oven used in industrial bakeries, small bakeries, or restaurants. A deck oven may consist of a single oven or multiple ovens stacked horizontally; each oven has individual temperature controls.

DHHS/FDA: Department of Health and Human Services, Food and Drug Administration.

Emulsifying agent: An ingredient having both polar and nonpolar groups, allowing for attraction of both polar (water) and nonpolar (oils) ingredients. Emulsifying agents improve keeping qualities of muffins by dispersing water throughout the batter.

FAO/WHO: Food and Agricultural Organization of the World Health Organization.

Fat replacer: Ingredients used to replace fat in baked products to meet consumer demand for "healthier" foods lower in calories and saturated fat. Fat replacers replicate the mouthfeel and keeping qualities of fat by attracting water.

FDA: U.S. Food and Drug Administration.

FDA/CFSAN: Food and Drug Administration/Center for Food Safety and Applied Nutrition.

FDA/ORA: Food and Drug Administration/Office of Regulatory Affairs.

Formula: Term used instead of "recipe" by the baking industry; the weight of each ingredient is determined based on the weight of flour at 100%.

FSA: Food Standard Agency of the United Kingdom.

FSANZ: Food Standards Australia and New Zealand.

Functional foods: Food marketed to have specific health benefits; for example, a health benefit of including oats in the diet is lowering blood cholesterol.

Gelatinization: Changes in the starch granules of flour (breaking of hydrogen bonds and swelling) in the presence of water and heat; starch gelatinization gives structure to quick breads.

GMO (genetically modified organism): Refers to new plant varieties developed using genetic engineering or biotechnology, and ingredients made from GMO plants, for example, cornmeal made from genetically modified corn.

Hard wheat: A type of wheat used for making flour with high or intermediate amounts of protein (11–14%) and gluten strength suitable for the production of bagels, sourdough breads, and other yeast breads.

Hydration: The addition of liquids to dry ingredients in the preparation of quick breads; hydration promotes starch gelatinization, which gives structure to the final baked product.

Hygroscopic: A quality of attracting water molecules; sugar in muffin batter attracts water and contributes to the moistness and keeping qualities of baked products.

Lactose: The disaccharide made of glucose and galactose and found in milk. Both lactose and protein in milk contribute to Maillard browning in baked products.

Maillard browning: A change in color that occurs during the baking process as a result of the reaction between an aldehyde or ketone group from sugar and the amino acids from protein sources in the batter such as milk, soy, and eggs.

Malt extract: An ingredient used in water bagels to promote crust browning. The liquid extract is obtained from malt by mashing at progressively higher temperatures until all starch has been converted to maltose and dextrin. The extract is concentrated to form a thick syrup with a color ranging from light amber to deep brown.

Mouthfeel: Refers to the textural qualities of food perceived in the mouth. Characteristics can be described as gritty, hard, tough, tender, light, or moist.

NLEA: Nutrition Labeling and Education Act.

Nutraceuticals: Naturally derived compounds from food, botanicals, and dietary supplements marketed to prevent disease or to treat specific medical conditions. For example, plant sterol esters are added to vegetable oil spreads; the health benefit of these spreads is lowering of serum cholesterol.

Neutralizing value: The parts of sodium bicarbonate that will be neutralized by 100 parts of a leavening acid such as monocalcium phosphate.

NV: Neutralizing value.

Organic: Term used on food labels to identify agricultural products produced under specific guidelines as defined by regulatory agencies, such as the U.S. Department of Agriculture National Organic Program and the Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission on Organically Produced Foods. Vegetables

are grown without using conventional pesticides, petroleum-based fertilizers, or sewage sludge-based fertilizers. Animal products identified as organic come from animals that are grown without the use of antibiotics or growth hormones, are given organic feed, and have access to the outdoors.

Rate of reaction: The percent of carbon dioxide released during the reaction between sodium bicarbonate and a leavening acid phosphate under standard conditions of temperature and pressure.

Reel oven: Ovens with a Ferris-wheel-type mechanism to move four to eight shelves in a circle, allowing baking pans to be moved to the front of the oven for removal.

Scaling: A term used by the baking industry to describe the weighing of ingredients, dough, or batter.

Shelf-life: The "keeping" qualities of baked products such as moistness and tenderness; sugar and fat extend the shelf-life of breads.

Sodium aluminum sulfate: A slow-acting acid, used in combination with a fast-acting acid such as monocalcium phosphate in double-acting baking powder, which acts as a leavening agent in quick breads.

Sodium bicarbonate: Commonly called baking soda, a leavening agent used in combination with acid ingredients such as sour cream, yogurt, buttermilk, or fruit juice in muffin batter. Baking powder includes both an acid salt (monocalcium phosphate) and an alkaline salt (sodium bicarbonate).

Sodium chloride: Commonly called salt, and added to baked products to enhance other flavors.

Sugar replacers: Calorie-free or reduced-calorie ingredients used in baked products to give sweetness with fewer calories than sugar; sugar replacers are used to meet the demands of consumers for "healthier" foods.

Trans fat: The form of fat in partially hydrogenated vegetable oil or "shortening", used in commercial bakery products. Diets high in trans fat raise LDL-cholesterol and increase the risk for cardiovascular disease.

Transite: A high-density, fiber-reinforced, cement sheet that can withstand very high temperatures up to 315°C. Used as a baking surface in ovens for baking bagels.

True formula percent (*true percent*): Term used by the baking industry to describe the amount of each ingredient by weight for a "recipe" or formula compared with the total weight of all ingredients.

Vital wheat gluten: The insoluble gluten protein of wheat flour that has been concentrated by removing starch and other soluble components and drying to produce a free-flowing powder that contains 75–80% protein and has a water absorption of 150–200%. Used as an ingredient in bakery products to strengthen and control expansion of dough to ensure a product of uniform shape.

Water activity: The ratio of vapor pressure in food compared with the vapor pressure of water. Meats and fresh fruits and vegetables have high water activity; the addition of salt or sugar to foods lowers water activity because both salt and sugar attract and hold water.

WHO: World Health Organization.

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16

Fundamentals of Cakes: Ingredients and Production

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16.1 Introduction 308
16.2 Ingredients 308
16.2.1 Flour 308
16.2.2 Liquid 309
16.2.3 Sugar 310
16.2.4 Shortening 311
16.2.5 Leavening Agents 311
16.2.6 Eggs 313
16.3 Cake Batters: Characteristics and Mixing 313
16.3.1 Shortened-Style Cakes 314
16.3.2 Methods for Mixing Shortened Cakes 314
16.3.2.1 Creaming Method (Conventional Method) 314
16.3.2.2 Conventional Meringue Method 315
16.3.2.3 Conventional Sponge Method 315
16.3.3 Baking 315
16.3.4 Cake Pans 316
16.3.5 High-Altitude Adjustments 316
16.3.6 Foam-Style Cakes (Unshortened Cakes) 317
16.3.6.1 Angel Food Cake 317
16.3.6.2 Yellow Sponge Cake 318
16.3.6.3 Chiffon Cake 318
16.3.6.4 Genoise Cake 318

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

16.4 Industrial Manufacturing of Cakes 319
16.4.1 Mixers 319
16.4.1.1 Vertical Dough Mixers 319
16.4.1.2 Continuous Mixers 320
16.4.2 Baking Cakes 321
16.4.2.1 Depositing the Batter 321
16.4.2.2 Heat Transfer in the Oven 322
16.4.2.3 Ovens 322
References 324

16.1 INTRODUCTION

The art of baking cakes dates back to the time of the earliest settled communities. Traces of small cakes, rather like modern cookies, have been found in Neolithic villages inhabited more than 10,000 years ago. The first cakes were probably baked around the time people discovered how to grind grain, thereby producing flour (Anon. 2003). Cakes gained in popularity once cooks had access to sugar. Cane sugar, which historians believe was first manufactured in India, traveled from India to Arab countries, where it was artfully combined with spices and flavorings imported from the Far East. By the seventeenth century, many cooks were using beaten egg whites to replace yeast in leavening cakes. The era of the light-as-air sponge cake had arrived. These European cakes form the basis of our cake heritage (Anon. 2003).

The two basic categories of cakes – foam and shortened – are distinctly different in their preparation and problems. The butter or shortened style of cake (pound cake, yellow cake, chocolate cake, and so on) is made with fat. Foam style cakes are made without fat and include angel and sponge cakes. Chiffon cakes are made with oil, but resemble sponge cakes.

The quality of a cake is dependent on variations of certain factors. However, there is much more to making an acceptable product. The main principle involved in a cake formula is to provide the proper proportions of the various ingredients so that the tenderizing agents – sugar, shortening, egg yolks, leavening agents, and chocolate – counteract the toughening or binding ingredients – flour, egg whites, and milk solids. The final step is adherence to optimal mixing and baking procedures. This chapter will deal with the different aspects of cake production, such as function of ingredients, mixing methods, and baking requirements, focusing on their effects on cake quality.

16.2 INGREDIENTS

Cakes have a higher proportion of sugar, milk, and fat to flour than do breads, and the flour used is usually cake flour. Both flour and eggs contain the proteins that contribute strength and structure to cakes (Corriher 2001).

16.2.1 Flour

Flour provides structure, texture, and flavor to baked products. Starch is one of the components in flour that strengthens the baked item through gelatinization (changes that starch undergoes when subjected to moist heat), and is one of the factors that contributes to crumb.

Crumb is partially created during baking by the number and size of air cells produced, the degree of starch gelatinization, and the amount of protein coagulation (Jacobson 1997).

For cakes the flour of choice is cake flour. It is pure white and has a fine, silky soft texture. Its protein content may vary from 7.5 to 8.8% for high-sugar cakes, and 8.5 to 9.5% for heavier cakes (Pyler 1988). Cake flour has a small particle size compared to all-purpose flour, resulting in less gluten formation, which gives the cake a fine grain, a delicate structure, and a velvety texture. In most cakes the protein should produce a soft gluten that does not toughen significantly during the mixing process, but is still strong enough to support the foam structure of the cake. Although gluten formation is important, the ability of the intact starch granules to gelatinize is more essential for cake structure. Because gelatinization is dependent on the presence of the necessary amount of water, the liquid-carrying capacity of cake flour should be as high as possible. The capacity is dependent on not only the protein content of the flour, but also on its maturing treatment and granulation (Freeland-Graves and Peckham 1996).

The maturing treatment of flour consists of two steps. The first step is chlorination, which decreases the pH of the flour from 5.8–6.1 to 4.6–5.1, bleaches the plant pigments, and improves baking quality (Fustier and Gelinas 1998). Chlorination increases the water absorbency of the starch, which contributes to a firmer, smaller crumb and a stable product. This is especially important with the high amount of sugar in the cake formula and, because sugar is a good competitor for water, chlorination aids the starch in this capacity (Conforti and Johnson 1992). Chlorination is also believed to increase the surface porosity of starch granules (Varriano-Marston 1985). The porosity of the starch permits increased uptake of liquid which improves starch geletinization. The second step in maturing is treatment with benzoyl peroxide. This treatment eliminates any traces of color left from chlorination and produces a pure white color.

All-purpose flour can be used to make cakes, but the cakes may have a slightly coarser texture and lower volume (Yamazaki and Donelson 1972). The protein content of all-purpose flour averages about 11%, but bread flours, which need strong gluten development, have between 12 and 14% protein. Blending hard and soft wheat flours yields flour that can be used for all purposes – breads, cakes, or pastry. Despite what appears to be a uniform product, the actual protein content of all-purpose flour may differ from region to region and brand to brand. Regional differences have influenced manufacturers to place more protein in flour sold in the northern United States where yeast breads are more popular, compared to the South, where biscuits requiring a softer flour are more routinely consumed. All-purpose flour may or may not be bleached.

Self-rising flour is usually all-purpose flour, but cake flour could also be self-rising. The leavening agent and salt are added to the flour. The leavening agent is a baking powder (baking soda combined with a salt, monocalcium phosphate). One cup of self-rising flour contains about $1\frac{1}{2}$ teaspoons baking powder and $\frac{1}{2}$ teaspoon salt.

16.2.2 Liquid

Whether water, milk, or buttermilk is used in the recipe, their function will be the same. Liquid hydrates the starch in the flour and, when heated, causes gelatinization of starch. Liquid dissolves the ingredients, especially sugar, during mixing and baking of the cake batter. It is also essential for the release of carbon dioxide from either the baking powder or baking soda. Liquid also produces steam along with carbon dioxide, which aids in leavening. Milk is a popular liquid to be used in cake batter. In addition to contributing water, milk adds flavor and nutrients (complete protein, B-vitamins, and calcium), and contains certain

compounds that help produce a velvety texture, a creamy white crumb, and a browner crust. The lactose in the milk participates in the Maillard reaction (see the section on Sugar), resulting in a brown crust. Non-fat dry milk (NFDM) is often added for the enhancement of a golden crust color because of the Maillard browning reaction of the lactose and proteins. This NFDM changes the structural characteristics of the cake because it decreases the swelling of the starch granules, but has no effect on cake volume (Pearce and others 1984).

The liquid portion in a cake recipe must be worked out with care; too little liquid could cause (1) a peaked center and/or a cracked top because the batter is too thick; (2) a dip in the center due to insufficient starch gelatinization; and (3) a dry cake that stales very quickly. Too much liquid can produce a cake with (1) low volume due to poor retention of air in the batter and (2) a very moist (soggy) heavy texture.

16.2.3 Sugar

Aside from contributing sweetness, sugar also influences the volume, moistness, tenderness, color, appearance, and calorie (kcal) content of baked products. One important function is that it increases the volume of cakes by the incorporation of air into the fat during creaming. During baking, sugar raises the temperature at which gelatinization and coagulation occur, which gives the gluten more time to stretch, thereby further increasing the volume of the baked product and contributing to a finer, more even texture (Bean and Yamazaki 1978). The hydroscopic or water-retaining nature of the sugar increases moistness of the baked cake. This is evident when brown sugar is used in the formula. Brown sugar is used in some low-fat products because of its hydroscopic nature, which, therefore, increases moistness in the cake. Sugar competes with starch for available water necessary for the hydration of proteins and starch and eventually gluten development. Finally, sugar helps to brown the crust through caramelization and Maillard browning. Caramelization is a process in which sucrose alone is involved in a series of chemical reactions caused by the baking temperatures (Camire and others 1990). Maillard browning involves two components: protein and carbohydrate. Flour contains protein and carbohydrate. Sugar, also a carbohydrate, participates in the reaction, which produces a brown crust (Whistler and BeMiller 1997).

Syrups of various kinds are used in cake batters, but these, too, produce a difference in the appearance and palatability of the baked product. For example, honey in a cake formula produces a product that remains moist for a longer period of time owing to its fructose content (fructose is a sugar that is highly hydroscopic). However, honey cakes are heavier and more compact than cakes made with crystalline sugar.

For special diets and products, alternative sweeteners are used in cakes. Only four alternative sweeteners are currently approved by the FDA for use in the United States: saccharin, aspartame, acesulfame-K, and sucralose. Two new sweeteners that have been approved by the FDA are alitame and neotame. In addition, cyclamates, which were approved but later banned in the United States as a potential carcinogen, are attempting a comeback.

One drawback of alternative sweeteners is that they do not provide the important functional characters of sugar: bulking, binding, texturing, and fermenting. However, certain compounds can be added to foods to compensate for bulking. These compounds include cellulose, maltodextrin (also used as a binding property), the sugar alcohols, and polydextrose. Polydextrose provides a texture similar to sugar, but the amount may have an adverse effect on texture in high-ratio cakes (cakes with a higher ratio of sugar and liquid to flour) (Pateras and Rosenthal 1992).

16.2.4 Shortening

Shortening is the primary tenderizing agent in the cake. Shortening also imparts moistness. A layer cake made with oil as the shortening gives the impression of a moister crumb than does a cake from the same formula made with an emulsified plastic shortening (Stauffer 1998). Keeping quality and, depending upon the type of shortening, flavor, round out the attributes. Pound cakes prepared with butter impart a flavor that cannot be equaled compared with when prepared with a plain shortening.

The hydrogenation process creates a plastic fat that is important to the creation of a cake batter that traps air bubbles to produce a fine-grained and high-volume cake. Cakes typically contain 15–25% fat on a batter weight basis. Therefore, high-ratio shortenings (containing mono- and diglycerides) are recommended. This type of shortening significantly enhances the structure of the cake batter, thus permitting the sugar-to-flour ratio to be increased and, consequently, improving cake quality (Wainwright 1999). Use of an emulsified shortening distributes the fat more evenly and finely in the batter (Moncrieff 1970). During mixing, fat and sugar are combined and mixed (creamed). During this step, the air is dispersed in the solid phase. In the presence of an emulsifier such as monoglyceride, these bubbles are divided into numerous small air cells by the action of the beater. The shortening must be solid so that the bubbles do not escape but also plastic so that it can fold around each air pocket (β' crystal structure of fatty acids in the fat).

When a one-stage process (all ingredients are added at once) is used in the mixing the air is entrapped in the water phase rather than in the shortening. To form the air-in-water foam, an α-tending emulsifier in the shortening is required. The most common emulsifiers that are used, at 7–10%, in the plastic fat are propylene glycol monoesters (PGME) and acetylated monoglyceride (AcMG) (Stauffer 1998).

Cakes made with oil as the shortening are more tender than those made with a plastic shortening. The cake gives an impression of moistness when eaten, even after storage for a week or more (Stauffer 1998). In commercial cake production, unfortunately, oil cakes are often too tender to withstand the necessary handling along the production line. However, tenderness is highly desirable from the consumer's standpoint, and so packaged cake mixes for home use are usually made with oil for the shortening. Again α-emulsifiers are used to stabilize the foam created by proteins contributed by eggs and flour. Propylene glycerol monostearate (PGMS) and stearic acid (80 : 20) help to form a strong film (Stauffer 1998).

Fat can be reduced in cakes, but the properties of the final product will be altered. Decreasing the amount of fat in a cake will create a lower volume and denser crumb with tunnels. Because the "ideal" fat substitute does not exist, a systems approach to reduced-fat or low-fat food formulations has been proposed. Simply put, a systems approach uses a combination of different ingredients that may or may not belong to either of the classes of fat replacers and requires a basic knowledge of ingredient technology to formulate desired products. The system may contain emulsifiers, fat substitutes or mimetics, fibers, water control ingredients, flavor, and bulking agents. Water or moisture control poses one of the greatest challenges in formulating reduced-fat snacks or baked goods. In these systems water is used to replace fat, to increase bulk, or for functionality (Akoh 2002).

16.2.5 Leavening Agents

Leavening agents are characterized as air, steam, and CO_2 (carbon dioxide). Chemical sources of CO_2 in a cake batter are baking powder and baking soda. These ingredients are essential for a risen product and will be described below.

The initial leavening agent incorporated into the cake batter is air. Simple manipulation of the ingredients and batter incorporates air. Air is incorporated by the simple action of sifting together the flour and other dry ingredients (salt, baking powder, and/or baking soda). In many angel-food-cake recipes the sifting of flour and sugar is recommended at least four times to ensure proper air incorporation. Another method that incorporates air is "creaming" of fat and sugar. The characteristic of the fat (plastic fat) helps in the incorporation and trapping of air and distribution of air cells into small units in the batter. During heating, air is released and the product rises. Beating egg whites, especially important for foam-style cakes, is another example of incorporating air into the batter. Egg whites warmed to room temperature are able to hold onto the air that is beaten into them. Whole eggs are beaten into a foam for some cakes (genoise, for example). Usually the eggs are warmed over a water bath and beaten at the same time in order to trap air during beating. Whether it is a whole egg or egg white, the foam must be baked immediately because if it stands for any length of time air is lost and so is the volume of the cake. Careful folding of the ingredients is a manipulation technique that must be carefully observed. Over-manipulation of the foam causes loss of air and a heavy, low-volume cake.

Steam is formed by the liquid in the recipe. Whether the liquid is water or milk, when heated steam is produced, the cake rises. Liquid has other functions in the recipe (see section on Liquid). Egg whites are made up of water ($\sim$88%). A foam-style cake, particularly angel food cake, relies on the steam produced by the egg whites. Therefore, a true foam-style cake is leavened predominately by air and steam.

In shortened cakes, the major part of leavening is done by a commercial baking powder or baking soda. When either of these compounds comes in contact with a liquid and heat, a chemical reaction occurs that produces CO_2, and the product rises. When a cake rises to the proper height, the texture is porous and light.

Baking soda (sodium bicarbonate) chemically yields CO_2 when it reacts with an acid in the recipe. Examples of an acid would be buttermilk, sour milk, cocoa, yogurt, sour cream, applesauce, honey, brown sugar (contains organic acids), and fruit juice. Up to $\frac{1}{4}$ teaspoon baking soda per cup of flour is recommended.

The two types of baking powder are fast, single-acting powder and slow, double-acting baking powder. The different types of baking powder all give off the same amount of carbon dioxide, but they have different effects on cake quality because of the distinctive tastes of their salts, different rates of evolution of carbon dioxide, and effects on stability of emulsions in the batter. A flour mixture made with fast or single-acting baking powder should be handled quickly and efficiently and placed in the oven as soon as possible, because it starts to produce carbon dioxide as soon as water is added. Any delay allows the carbon dioxide to escape and decreases the ability of the mixture to rise. Almost 75% of the carbon dioxide is evolved in the mixing of the batter when a single-acting baking powder is used, compared with less than 50% with a double-acting baking powder. The greater loss of carbon dioxide before baking when using single-acting baking powder can decrease the volume of the baked cake. Approximately $1\frac{1}{2}$ to 2 teaspoons of single-acting baking powder are required for every cup of flour.

Many commercial bakers use double-acting baking powder (sodium aluminum sulfate[SAS]-phosphate powder), which reacts twice: once in the mixing bowl and then in the oven (heating). Approximately 1 to $1\frac{1}{2}$ teaspoons of double-acting baking powder is required for every cup of flour.

16.2.6 Eggs

Eggs are added to help strengthen the structure, as well as to increase leavening, to act as emulsifiers, and to add color and flavor (Brown 2004). It is important to increase the amount of liquid as the amount of egg increases; otherwise the overall volume will decrease. Because eggs have a toughening effect, the addition of eggs is normally balanced by an increase of tenderizing agents – fat and sugar. Too much egg produces a tough product; too little may cause an irregular or coarse crumb.

Eggs are available in shell form, frozen, and dried as a powder. Unless treated, shell eggs will only keep a limited time, as the shell is porous and the membrane lining is semipermeable. Bacteria are able to penetrate to the interior of the egg, and as the egg is broken down by the bacteria, strong-smelling gases are given off (Whiteley 1971a).

Egg yolks are particularly rich and would enhance the flavor and appearance of most cakes. The yolk is rich in lecithin, which is valuable for its emulsifying properties in batters. Yolks are available both frozen or dried. Frozen yolks are either treated with sugar or salt to prevent any break down of the yolk after it is defrosted.

Egg whites are available because of their ability to form a foam. It is easier to whip frozen egg whites to a foam compared with fresh egg whites that have been pasteurized (either frozen or dried), and dried whole eggs that have been reconstituted do not whip to a foam as easily as their fresh counterparts. The foaming ability is reduced by the heat of pasteurization of the egg whites and by the partial disruption of the emulsion of fat in the yolk for the whole eggs. The foaming ability of dried whole eggs can be improved by whipping them at 140°F (60°C) (Freeland-Graves and Peckham 1996).

Sugar is added to the egg-white foam to form a meringue. The addition of sugar stabilizes the foam. When beating egg whites, the whites go through four stages: foamy, soft peak, stiff peak, and dry. At the dry stage the egg whites become dry and brittle and at the same time the cells become overstretched. When this occurs it is difficult to fold in the ingredients, and the cake will not rise. Usually the cells are overstretched and will collapse. Some cakes, such as angel food, call for the addition of sugar to the egg-white foam. When sugar is added, a meringue is formed (this is known as the meringue method). Cream of tartar is added to the egg whites during the foamy stage when the whites, are being whisked. Cream of tartar is an acid, and lowers the pH of the whites, thus stabilizing the whites. Sugar is added during the soft peak stage (peaks fall over when beaters are removed). Sugar is added gradually and then beating continues until the peaks are stiff and shiny. Sugar stabilizes the meringue, and there is no danger of overbeating the whites.

Demand for low-fat and low-cholesterol products has motivated the food industry to find substitutes for eggs in cakes. As eggs are used for the emulsification properties of lecithin, soybean lecithin can be substituted. Its advantage is that it contains no cholesterol, as soybean originates from a plant. A better approach is to use concentrated blends of the phospholipids present in eggs, such as phosphatidyl choline and ethanolamine. These blends of phospholipids create very soft and tender cakes (Silva 1990). The level of fat may be reduced 40–60%, because the phospholipid blends have the ability to complex with sugar and fat.

16.3 CAKE BATTERS: CHARACTERISTICS AND MIXING

Accurate measurement of the ingredients will help to guarantee a quality product. In the home, the use of correct measuring utensils and techniques will ensure an acceptable

product. On a larger scale, weighing of the ingredient is a more accurate way to prevent any mishaps in the cake formula.

16.3.1 Shortened-Style Cakes

Shortened-style cakes are the most commonly prepared cakes, especially for birthday and wedding celebrations. The modern shortened-style cake recipes have a higher sugar content for use with shortenings that contain mono- and diglycerides. Rules for these recipes have been generalized: (1) the weight of the sugar should exceed that of the flour; (2) the weight of the eggs should exceed that of the fat; and (3) the weight of the liquid in the eggs and milk should equal or slightly exceed the weight of the sugar.

The weight of sugar varies, as was established earlier. In yellow and white cakes, the sugar to flour ratio generally does not exceed 3 : 2 or fall below 1 : 1. Batters that have appreciable amounts of chocolate or cocoa may have a sugar to flour ratio higher than 3 : 2. Too much flour is undesirable as it can produce a heavy, dry cake with tunnels in the crumb. Tunnels can also form in excessive baking temperatures as well as from overmanipulation.

Because eggs have a toughening effect on batter structure, an equal or almost equal weight of fat must be used to provide a tenderizing effect. The term liquid refers to the total weight of the liquid ingredient and eggs. In a high-sugar-ratio cake made with emulsified shortening or fluid cake shortening, the amount of milk or liquid may be higher than in other recipes, and the combined weight of the liquid ingredients may exceed the equal-weight rule, rising as high as 165% of the flour weight.

The structure of the plain shortened cake is that of an air-in-fat foam distributed in a flour-in-liquid mixture. Starch is suspended as lumps in the batter; air, which is incorporated during the initial mixing, appears near the fat/starch pools. The presence and dispersion of the air bubbles is essential and can be regarded as the nucleus for the expansion of the cake. The complete dispersion of the ingredients is also a fundamental requirement for a good cake. Therefore, the purpose of batter mixing is (1) to disperse all the ingredients as efficiently as possible, and (2) to incorporate air into the mix. The incorporation of air takes place during (1) a period of rapid incorporation in the form of large bubbles, and (2) a stabilizing period when the bubbles are reduced in size (Bennion and Bamford 1973).

Fat melts and releases the air bubbles into the aqueous phase of the batter, the air and carbon dioxide expand throughout the batter, water is changed to vapor, and the fluid batter is lightened until the starch gelatinizes. The solid porous structure of the crumb is set by heat gelatinizing the starch and denaturing the proteins of the eggs and flour.

16.3.2 Methods for Mixing Shortened Cakes

16.3.2.1 Creaming Method (Conventional Method). The creaming method initially combines the shortening with the granulated sugar at slow to medium mixing speed until the components are thoroughly blended and the mixture has become aerated. This stage is followed by the incorporation of the eggs, and the creaming action is continued. Mixing is completed with the addition of milk and flour in alternate small portions (Pyler 1988).

Among the major advantages of the creaming method are the incorporation of large volumes of air in the form of minuscule cells in the fat phase of the batter: the

coating by the fat on the flour and sugar, which delays their respective hydration and solubility; and the near absence of flour gluten development. In the case of the current flours in the market, the last benefit no longer possesses the significance it formerly had, because these flours are bleached and have a low protein content.

Mixing time is important. Overmixing will cause a loss of air and a heavy cake. Total mixing time for the creaming method will range from 15 to 20 minutes: the initial creaming stage takes 8–10 minutes; the second stage with the incorporation of the egg, 5–6 minutes; and the final stage of milk and flour addition, 5–6 minutes. If the milk is added too quickly it will cause curdling of the batter and cause the emulsion to be inverted to a water-in-oil emulsion. If the egg whites have been separated from the yolks, they are beaten until stiff and folded into the batter at the very end. This is a time-consuming method for mixing a cake, yet it is a popular household method because it produces a fine-grained cake with a velvet crumb.

16.3.2.2 Conventional Meringue Method. This is a modification of the conventional method. It differs from the aforementioned method in that the separated egg whites and part of the sugar may be made into a meringue and folded into the batter at the very end.

16.3.2.3 Conventional Sponge Method. In this method the eggs are separated and about half the sugar is added to them. The sugar–egg mixture is beaten until foamy and stiff and then added to the other ingredients at the end of mixing. The advantage of the conventional meringue method and the conventional sponge method is that less fat is creamed with the sugar and more air is added at the end of mixing.

Quick Mixing Methods. Cakes made by the quick mixing methods are formulated to be made with emulsified shortenings. These are the high-sugar-ratio cakes. All the dry ingredients are sifted together in a bowl and then the fat, milk, and flavoring are added. After the mixture has been beaten vigorously for 2 minutes by an electric mixer, the eggs are added and the mixture is beaten for another 2 minutes.

SINGLE-STAGE (DUMP METHOD). This method is closely related to the quick method. This method consists of placing all the ingredients into a bowl and mixing them until they are combined.

PASTRY BLEND METHOD. In the pastry blend method the fat and flour are creamed together to produce a foam. Into this a mixture of the sugar, salt, baking powder, and one-half of the milk is blended; finally, the egg and the remainder of the milk are mixed in.

16.3.3 Baking

The batter should be baked as quickly as possible after being mixed. Cake batters that are not to be baked immediately should be covered tightly with plastic wrap and refrigerated until the batter is to be placed in the oven. Once the baking powder has entered the solution, some carbon dioxide is evolved. If the mixture is left standing for prolonged periods, some of the carbon dioxide will escape and the baked product will have a characteristically coarse cell structure.

During baking there is considerable movement of the batter by convection currents. Development of these currents is dependent on the temperature, batter type, and pan shape. Batters of high viscosity, such as pound cake, exhibit much less movement compared to batters of a low viscosity, such as high-ratio cakes. As the batter is heated, its viscosity decreases, as would be expected. However, the higher temperature also triggers the leavening system and produces gas. As the bubbles increase in size, the batter becomes a foam. The foam results in a higher apparent viscosity of the system. As the temperature of the system increases, the starch gelatinizes, and much of the free water becomes bound. This sets the cake into a rigid system that does not collapse as it cools. The top center of the cake is the last to bake. If the cake has too little or too much water, a dip forms at the center. With the correct amount of water, a smooth contoured crown is produced (Trimbo and Miller 1966, 1973).

16.3.4 Cake Pans

Cake pans are available in a variety of shapes and forms, ranging from flat sheet pans to cupcake molds to form-pans of various configurations. Pans for larger product sizes are generally fabricated as individual units, whereas pans for snake cake items are normally strapped into sets or consist of frames containing rows of individual cups or molds.

The weight, or gauge, of the pan's metal also affects quality – the heavier the pan the better. Crumb formation is partially dependent on the degree of heating that occurs when the cake batter is first placed in the oven, and rapid heat absorption plays a role in this. Cake pans have shiny sides with dull, rough bottoms. The shiny sides will reflect radiant energy, so that the batter will rise before the cake sets, and the dull rough bottoms will absorb radiant energy to form the bottom crust.

The pans are prepared prior to mixing the batter. For a shortened-style cake the bottom is greased, but the sides generally are not. The ungreased sides provide traction allowing the rising mixture to reach full volume. When greasing the bottom of the pan waxed or parchment paper may also be placed in the bottom before greasing to allow for easier cake removal.

To avoid cake hanging over the side, the pan should be filled only half full with batter before the cake is baked. If an odd-shaped pan is to be used the capacity can be measured with water, then only half as much batter is spread in the pan.

Cakes are done if the top springs back when lightly touched with a finger or when a cake tester inserted into the center of the cake comes out clean. The cake should not be tested until the minimum baking period is over. If the oven door is opened during the early stage of the baking time, complete gelatinization of the starch has not occurred, and the cake will collapse.

Cakes should be allowed to stand for 5–10 minutes or until the interior reaches 140°F (60°C) before they are removed from the pan. The cooling-off period permits the interior of the cake to become firm.

16.3.5 High-Altitude Adjustments

Cake ingredients must be modified at altitudes higher than 3000 ft. The lower atmospheric pressure at higher elevations reduces the need for baking powder, or baking soda. This affects the leavening agent because the volume of gas obtained from steam, air, baking powder, or soda expands with increases in altitude. If there is no mobilization, the reduced pressure of the atmosphere at higher altitudes presents less resistance to

expanding gases, and the cell structure of the cake becomes overstretched. This overstretching results in a coarse texture with large cells, which may cause the cake to fall. Also, at higher altitudes, water evaporates more quickly and the concentration of sugar increases. Structural strength can be improved by

1. Adding 1 to 2 tablespoons of cake flour;
2. Increasing the amount of liquid; or
3. Reducing baking powder and/or baking soda and sugar quantities.

Increasing the baking temperature 15–25°F (8–14°C) will help the protein structure denature before the expanding gases overstretch the gas cell walls.

16.3.6 Foam-Style Cakes (Unshortened Cakes)

Foam-style cakes include angel food and sponge cakes. Foam-style cakes depend on eggs for their structure and volume. This is dependent on the egg's ability to occlude air and to form stable foams. Typically, as the egg white is beaten or whipped, air is incorporated, and the air cells become increasingly smaller as beating continues. Also, as beating continues, the foam loses its sheen and reaches its maximum volume and stiffness.

16.3.6.1 Angel Food Cake. Angel food cake should be very light–almost fluffy. The cake is leavened only by air and steam, with no chemical leavening. The air and steam are primarily derived from the egg whites. Egg whites constitute the main ingredient along with sugar. Sugar is the tenderizing agent for the cake because the cake does not contain shortening. The major proportion of egg whites to sugar is 42 : 42 and flour is 15%. Cake flour plays a secondary role.

The lightness and volume of an angel food cake depend to a large extent on the method of combining ingredients. The goal in mixing is to completely blend the ingredients with the beaten egg whites without losing the air held by the egg foam.

In commercial practice, the general procedure in preparing angel food cake is to first whip the egg whites to which have been added salt and cream of tartar. Cream of tartar is considered an acid and a whipping aid for the egg-white foam. In addition, cream of tartar helps to whiten the cake batter by its effect on the flavonoid pigments of the egg whites, produces a fine-grained cake by stabilizing the proteins in the batter, stabilizing the foam sufficiently so it will not collapse in the oven before the temperature of coagulation is reached and prevents the drastic shrinkage of the foam during the last stage of baking and during subsequent cooling (Bennion and Scheule 2004).

Once the egg-white foam starts to form soft peaks, 50–60% of the sugar (of fine crystallinity to promote rapid solution) is added in a slow stream, while whipping is continued until the meringue retains a stiff peak. The flavor materials are then added, followed by the remainder of the sugar and the cake flour, which have been sifted together. Careful addition of the flour/sugar mixture is imperative. This mixture is added using the lowest speed on the mixer, or "folded in" by hand, in which a rubber spatula is used to cut down vertically through the two mixtures, across the bottom of the bowl and up the nearest side. The bowl is rotated a quarter turn with each series of strokes. The down-across-up-and-over motion gently turns the mixtures over on top of each other, combining them in the process.

The browning of the crust is not as dark as with other cakes. The Maillard browning reaction is not as intense due to the acidic pH of the batter caused by the cream of tartar. The color of the crust is characterized as "macaroon" in color, which is crisp and more sugary than that of a shortened cake.

Angel food cakes are usually baked at a high temperature (375–400°F; 191–204°C). Also, the majority of angel food cakes are baked in ungreased tube-style cake pans, which provide a more efficient heat distribution within the batter, as well as an additional internal temperature that promotes cake volume expansion.

16.3.6.2 Yellow Sponge Cake. The yellow sponge cake does not differ much from angel food cake except that the yolks as well as the whites are used. In some sponge cakes, a small amount of water may also be used. In making a sponge cake the goal is to reduce the toughening effect of the whole eggs by including a sufficient amount of sugar.

The making of sponge-cake batters may be carried out in a variety of ways. In some instances, bakers prefer to separate the white and yolks of eggs and beat them separately with the portion of the sugar to the desired specific gravity before recombining them. The aim of the approach is to attain maximum batter volume. Another procedure, however, is to beat the whole eggs, which have been tempered to a temperature of about 80°F (27°C) with a wire whip or blade beater at medium speed. Remember that the size of the bowl is important; for example, if three whole eggs are used, then use a 1 qt. bowl for beating so that the eggs are confined to a smaller area and will incorporate more air. Sugar is added at the onset of mixing or in a slow stream during beating. After the egg foam has achieved the proper volume, flour and liquid are folded in as lightly as possible to avoid breakdown of the foam. If shortening or butter is to be added, first it is melted and folded in at the end to minimize loss in volume. This procedure is desirable when preparing a small recipe.

In the egg separated method, the egg yolks are beaten. The sugar, salt, flavoring, and water are added, and the whole mass is beaten until very stiff. The flour is folded lightly into the mixture, after which the stiffly beaten egg whites (stiff, but shiny and not dry looking) are folded in (Briant and William 1956).

16.3.6.3 Chiffon Cake. Chiffon cake has some of the characteristics of cakes made without fat, yet oil is one of the ingredients used. The large quantity of eggs used in the basic recipe imparts to the cake the lightness characteristic of sponge cakes. The method of combination includes that the dry ingredients are sifted together in a bowl and a well is made in the center. To this mixture, the oil, egg yolks, liquid, and flavoring are added and the whole is combined until the mixture is blended. The egg whites and cream of tartar are beaten together until the whites are stiff and then folded into the flour mixture. Chiffon cakes are baked in an ungreased tube pan and are tested done when they spring back when touched with the finger. It is cooled upside down just like angel food and sponge cakes.

16.3.6.4 Genoise Cake. This rich light cake is made with cake flour, sugar, eggs, butter, and flavoring. It is similar in texture to a moist sponge cake. Because the genoise cake contains clarified butter, it can be considered a relative to the chiffon cake. Clarified butter is known as drawn butter. This butter has been slowly melted, thereby evaporating most of the water and separating most of the milk solids (which sink to the bottom of the

pan) from the golden liquid on the surface. After any foam is skimmed off the top, the clear (clarified) butter is poured or skimmed off the milky residue and used in cooking.

Preparation of the genoise batter is similar to the whole-egg sponge method. Traditionally, the eggs are placed in a bowl and tempered over simmering water until a temperature of 80°F (27°C) is reached. The eggs are then whisked or beaten with a mixer until light and fluffy (tripled in size). Sugar is beaten in gradually along with the flavoring. Cake flour is sifted prior to addition to the batter and then folded gradually into the mixture. Approximately, $\frac{1}{2}$ cup of the batter is added to the clarified butter and mixed lightly, but thoroughly mixed. This mixture is then folded into the batter. In an alternative method, the clarified butter alone is folded into the batter. Either way this step must be done swiftly but gently, because the fat could deflate the batter, air will be lost, resulting in a heavy product.

16.4 INDUSTRIAL MANUFACTURING OF CAKES

16.4.1 Mixers

Less energy is needed in the efficient mixing of cake batters. The type of mixer that is used requires the enfolding of air into the batter and subdividing the air into small bubbles.

Generally, the same type of mixer can be used for the shortened-style and foam-style cakes. The only factor that would be different would be the agitation that would be needed for the incorporation of the ingredients and this is accomplished by using specialized agitators and different speeds. Both batch and continuous mixers can be used for nearly all types of cake batters.

16.4.1.1 Vertical Dough Mixers. Vertical dough mixers, a class identified by the perpendicular orientation of the mixer shaft, are used widely in the food industry. A feature common to most vertical mixers is the use of removable bowls. Their other characteristics may be quite diverse; for example, there may be one or more beater shafts. The beater shafts may move in a planetary action or remain stationary, and the design of the agitators may be varied over a wide range. The types of vertical mixers of chief interest are the planetary mixers, which are capable of preparing most batters and some doughs and often are used for adjuncts such as icings, and the spindle mixers, commonly found in cookie and cracker factories (Matz 1992).

Planetary mixing for bakery products has been the world standard for many years. This method has now been perfected in the complex planetary mixing system design. The addition of a second tool inside the bowl permits product mixing with far superior results, greater versatility, and reduced mix time. The continuous bowl scraper that is used is a most important feature because it constantly scrapes the bowl's sides and bottom. This greatly reduces the mix time. It is no longer necessary to stop the mixer to scrape the bowl, because all the batter or dough is being constantly remixed, therefore yielding the best and most consistent product.

The bowl sizes for the mixer may be obtained as 20, 40, 80, 120, 140, 160, and 340 quarts. Even smaller sizes are available for home kitchens, bakeries, laboratories, and so on. Using the usual two-stage addition for layer cake batters, a 340-qt mixer can complete four 400- to 550-lb batches in an hour. The amount per batch depends largely on the specific volume reached. Whipped-type cakes prepared with wire whips (sponge, chiffon, angel food), require smaller batches (Matz 1992).

In large modern mixing installations a computer is utilized in the operation of the mixer. In fact, the mixing bowl can be automatically loaded while the cycle is under constant computer control. The computer permits memory storage of many of the recipes, which can be recalled and re-run by recipe number without expert baker supervision. Ingredients may be added at any stage of the mix cycle because the computer controls the entire cycle.

Pressure-bowl mixing is a method of maintaining a constant amount of air in the batter. Traditional mixing systems have only ambient pressure (room air) available for whipping or mixing. With a pressure bowl, the bowl is sealed at the top. Whenever a light batter is desired, instead of whipping for a longer time, or at a higher speed, an increase of gauge pressure to around 10 psi is made in the bowl. This results in small bubbles being generated by the wire whip and dispersed throughout the batter. At the end of the cycle, the air pressure in the bowl is released, which causes the bubbles to expand to about twice the previous size. A small amount of additional mixing will break up these bubbles into a uniform size while evenly dispersing them throughout the batter. With a controlled amount of air this will yield the same specific gravity for the batter.

16.4.1.2 Continuous Mixers. Semicontinuous mixing of cake batters was introduced in the United States in the mid-1940s. One of the first commercial continuous cake-mixing systems was designed around a votator scraped surface mixer whose design is based on an ice-cream freezer machine. Another early system used a modified Oakes marshmallow mixer. In both plants, the mixer operated continuously but was fed with a premix prepared batchwise in a standard batter mixer. About 1950, a truly continuous cake-mixing plant based on the Oakes mixer was designed and installed. Since then, other manufacturers have introduced similar mixers.

Continuous batter mixers can be incorporated into completely continuous systems if production schedules warrant, as in plants making only a few varieties, and those in long unbroken runs. In such plants, liquid ingredients and plasticized shortening are premixed in vertical tanks equipped with high-speed propeller agitators. The resultant emulsion is metered into an open vortex-type premixer to which flour is fed by a gavimetric feeder. The premixer forms a slurry that is metered into the continuous mixer along with a stream of air.

Cake batter is much easier than bread dough to transfer through pumps, pipes, valves, and meters, so the engineering for continuous production is easier for a cake plant than for a bread bakery. The economics of cake manufacture is such that expenditure of a large amount of money for continuous-mixing equipment may not be warranted, however, particularly for small- to medium-sized plants handling a large number of different formulas (Matz 1992). There are basically two types of continuous mixers: the barrel type, which is suitable for mixing doughs, and the rotor and stator head type, which is suitable for fluid mixings such as batters and marshmallows.

The Oakes continuous automatic mixer of the rotor and stator head type is suited for the production of marshmallow and batters, such as cake and sponge. The mixing head consists of three parts, rear stator, rotor, and front stator. The stators are bolted together, with the rotor mounted on a revolving shaft between the two stators. The mixing head is small, measuring up to 14 in. diameter and 2 in. depth. The head is surrounded by a water jacket to facilitate temperature control. The internal faces of the stators and both faces of the rotors are provided with concentric rows of blades, which intermesh, with ample clearance, when the rotor is in motion. Provision is made for the prepared syrup, or batter,

together with a supply of air under pressure, to enter the mixing head through the center of the rear stator and flow between the blades of the rear stator and rotor to the outer circumference of the stator cavity. The mixture then flows between the blades of the front stator and rotor to the discharge point in the center of the front stator. The mixing action is very precise, and can be readily controlled by rotor speed, syrup or batter flow speed, and air pressure, to produce the required specific gravity of the mixing. The aerated mix is very stable and has a smooth and uniform texture. As the mixing head is small (the largest has a capacity of less than 3 qt.), the specific gravity can be rapidly adjusted, and when changing color and/or flavor of a similar mixing, very little waste between mixings will occur. The stainless steel head can be stripped down for inspection and/or cleaning in a very short time. The Baker Perkins Homogenizer or continuous batter mixer, consists of a two-section mixing head, with a rotor and triple impellers in the inlet section, and the main section contains three more impellers, situated between pierced static plates, through which the batter is forced (Whitely 1971b). The Oakes continuous mixer takes advantage of a state-of-the-art, automatic specific-gravity control system.

The AMF mixer is similar in external appearance to the Oakes, but the mixer head is considerably different. Instead of splitting axially for dismantling, the stator separates radially. The rotor has several rows of teeth on its periphery. These teeth mesh with rows of teeth on the inside of the stator wall. Rotor speed is continuously variable. The mixers are equipped with a batter metering pump, air meter, and speed controls (Matz 1992).

The Goodway mixer/foamer has a slightly conical mixing chamber, pyramidal teeth, thermal jackets, and a working pressure of 150 psi. The Fedco mixer has a cylindrical chamber several inches long with hundreds of square-tipped teeth. It encloses a cylindrical rotor having many teeth on its periphery. Flow of product is straight through the annular space. The intense shear applied to the batter in mixing heads of this design is sufficient to emulsify air and shortening in a complete formula blend. Conventional batch mixers often require that the water be kept back until the shortening has been creamed with some of the dry ingredients. Their principal advantage, however, lies in the continuous mixer's unique ability to produce highly aerated batters such as angel cake batter from a rough premix. Air or inert gas is metered into the mixer head at a calculated rate (Matz 1992).

16.4.2 Baking Cakes

16.4.2.1 Depositing the Batter. Cakes and other products made out of fluid batters assume the contours of the containers in which they are baked, so the forming or shaping machinery may be regarded as the pans themselves, along with the pipes, pumps, and metering devices that deliver batter to the pans. Finishing equipment that enrobes, deposits, injects, spreads, or otherwise adds fillings, toppings, and so on, has very important effects on the appearance, cost, and nutrition of the final products.

Batter depositors may be either manifold-type or volumetric hopper-type. Accuracy is a prime consideration. Ability to handle batters containing pieces such as raisins, nuts, and chocolate chips without reducing their size is important. The depositor should not markedly change the specific gravity established at the mixer. Sometimes the viscosity of the batter is adjusted to allow flow into all parts of the pan. Vibrating the pan strongly during and after depositing the batter is of considerable help, but it may bring all the gas bubbles to the surface, with unfortunate effects on the appearance of the cake.

Whether deposited on the oven band or in open pans, the top surface of these cakes will be flat or slightly domed.

16.4.2.2 Heat Transfer in the Oven. Whether a gas or electric oven is used in baking a cake, heat is produced. This heat energy is transferred to the food through conduction, convection, and radiation when using a conventional oven.

Conduction can take place in solids, liquids, and gases, but cannot take place in a vacuum where there is no matter present to transfer the energy. It is the only way heat can be transferred in solids. Transference of heat by conduction can be illustrated by using a poker. If the end of a poker is placed in a fire, the other end soon becomes hot; the heat has been transmitted by conduction from one end of the poker to the other. Good conductors are used for hot plates, saucepans, shelves and sides of oven, and for baking trays and tins, so that heat is easily transferred to the bread or confectionery being baked (Dean and Russell 1980).

Convection has been defined as "the movement of a body carrying heat with it". Convection currents play a very important part in the heating of ovens. The heated air and flue gases circulate all around the ovens by convection currents, and in order that they should give up as much of their heat as possible before going out into the atmosphere, baffle plates are fitted to help the gases to circulate. These are metal plates with holes in them (Dean and Russell 1980).

The third manner of heat transfer is radiation. Radiation is defined as "the transference of heat from one body to another, without the intervening space being affected". A dull black surface is preferable for the inner walls of an oven, as they are required to radiate as much heat as possible. New baking trays are often put in the oven for some time before use, in order that their shininess should wear off; they will then absorb radiated heat better (Dean and Russell 1980).

The conventional oven relies on hot air for heating food, primarily by convection, but conduction and radiation can also occur. Baked goods rely on freely moving currents for the transfer of heat, so it is important to ensure that baking pans are placed on racks in such a way as to allow for the efficient flow of air currents. The position of the rack in the conventional oven is important to promote an even cooking and browning of the cake. For layer cake pans the rack should be in the middle or the lower third of the oven, and for a tube pan the rack should be in the lowest position, so the top of the pan is centered in the middle of the oven for even browning.

In the convection oven, hot air is circulated by a fan. Therefore, baking is accomplished more quickly. Usually, the position of the rack is not an issue because there is an even distribution of heat during baking and no hot or cold spot should exist depending on the quality and make of the oven. As the convection oven is a more efficient way to cook food, the temperature is lower by 25°F (−4°C) than the recipe suggests. Also, cooking time is less (as much as 25% less) than it would be in a conventional oven, even with the 25°F reduction (Middleton 2000).

16.4.2.3 Ovens. There are many types of ovens in use, and it is difficult to state which types are the best, but for confectionery work there has been, for years, an increasing tendency to use either gas or electricity as fuel, because they are clean and involve the lowest labor cost in their operation. Ovens may be classified in various ways, according to their construction, method of use, and method of heating.

Reel Oven. Reel ovens are really hot boxes, heated for the most part by gas, within which a set of 6–8 swing tray carriers revolve slowly about a central axis. The carriers are

stabilized and the baking sheets are loaded and unloaded at the same point. One advantages of this arrangement is considerable saving in space compared with peel ovens, which of necessity require a large peeling area for loading and unloading, which is an uneconomic use of floor space. An indicator on the front of the oven identifies the shelf that is passing the door at any given time, so that the operator can stop rotation and add pans to, or remove them from, specific shelves. They are very suitable for the baking of morning and other small goods and also for slab cakes.

Rotary Rack. The oven is a hot vertical box into which whole racks of goods are loaded. They have one- or two-rack capacity. The ovens are either electro-heated, oil- or gas-fired. The oil or gas burner is a high-pressure type suited to the oven and fitted with automatic temperature control and flame failure devices. The transmission of heat is by forced-air convection. The whole volume of air in the oven is recirculated many times per minute, passing through a compact heat exchanger unit and then horizontally across the chamber through the rack or racks on which the baked goods are set on standard baking sheets. During baking the racks are rotating continuously, thus ensuring even heating across the trays. The racks take up to 18 or 20 trays each according to the finished height of the goods being baked, and can accommodate mixed batches providing the articles require similar temperatures and baking times.

Band Ovens. The characteristic feature of a band oven is the continuous steel belt that forms the baking surface and which turns around two large metal drums, one at each end of the oven. There are many advantages to this. The baking chamber can be made very long (300 ft is a fairly common length), leading to fast transit times and high production rates. The band could be extended beyond the oven entrance sufficiently to accommodate certain forming operations or it could be extended past the exit to allow a modest amount of cooling and some additional drying so that the product does not have to be removed from the belt until it is set, thus providing support for the piece during the whole period of its maximum fragility. There is no need for elaborate transfer mechanisms. The baking chamber itself can be restricted in volume to almost the minimum theoretical space required to hold the product and the hearth so that the heat control is simplified and heat loss reduced.

Baking cakes as a continuous ribbon on an oven band has become common in large installations. Individual cakes are formed by cutting pieces from the baked strip and combining them in several ways. In manufacturing cakes, batter is prepared in a continuous mixer and pumped to a manifold, or batter distributor, positioned above the band oven. The manifold spreads the batter to a uniform depth, although more than one strip may be placed on the band if baked edges are preferred to cut edges, as they may be when preparing Swiss rolls (Freihofer 1985).

In addition to restricting the shape of the finished products, baking cakes on a band also establishes limits on formulation. It is important that the batter does not spread significantly during baking. Sponge-cake batters of high specific gravity are preferred. The finished cakes are often lower in moisture than is considered satisfactory for conventional cakes.

Travelling. Most confectionery traveling ovens are of the horizontal tunnel type. The oven chamber, divided into a number of zones, is a long tunnel up to 200 ft in length. Transmission of the goods through the oven is carried out in a number of ways, each of which is best suited for different types of products:

1. A continuous linked chain on which baking sheets are placed, which is suitable for a very extensive range of small goods;
2. A continuous band consisting of fairly heavy metal plates, which is suitable for the transmission of heavier products, such as slab cake; and
3. A flexible band on which a continuous stream of the mix is spread. This is the type universally used for baking swiss roll. It is also suitable for drop mixings, deposited direct onto the band.

Tunnel ovens are the most commonly used oven type in the cake-baking industry because they meet the baker's major concerns, such as minimization of energy consumption and an increase in mass production (Baik and others 2000). Compared to the batch oven, a predominant characteristic of tunnel-type ovens is that the whole baking chamber is divided into several zones along the oven length. In each zone, the temperature of the upper and lower baking chamber can be independently controlled, so the application of a temperature sequence is possible. This gives the baker more flexibility in adjusting conditions to the optimum values for a given product. However, baking phenomena in tunnel ovens are more difficult to predict than in batch-type ovens, usually operating at a constant temperature. The baking chamber of tunnel-type multizone ovens exhibits wavy temperature profiles along its length (Baik and others 2000).

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17

Traditional Italian Products from Wheat and Other Starchy Flours

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17.1 Introduction 328
17.2 Pasta 329
17.2.1 A Brief History 329
17.2.2 Dried Pasta 330
17.2.2.1 Definition of the Product and Quality Characteristics 331
17.2.2.2 Production Technology 334
17.2.3 Fresh Pasta 339
17.2.3.1 Classification Criteria 340
17.2.3.2 Quality Indices 342
17.2.3.3 Raw Materials 343
17.2.3.4 Pasta-Making Process 344
17.2.3.5 Packaging 350
17.2.4 Heat Damage to Pasta 352
17.3 Bread and other Savory Products 354
17.3.1 The Raw Materials 354
17.3.1.1 Wheat Flours and Flours from Other Cereals 354
17.3.1.2 Yeast 356
17.3.1.3 The Other Ingredients 358
17.3.2 Bread-Making Process 359
17.3.2.1 Bread-Making Processes with Baker's Yeast 359
17.3.2.2 Bread-Making Processes with Sourdough 361

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

17.3.2.3 Optimization and Innovation of the Sourdough Process 363
17.3.3 Process Card of Typical Italian Savory Products 366
17.3.3.1 An Example of the Sourdough Process: Pane di Altamura 367
17.3.3.2 Examples of the Straight and Dough Process: Grissini and Taralli 368
17.4 Conventional and Typical Italian Products from Corn 370
17.4.1 Wet Milling 372
17.4.2 Dry Milling 373
17.4.2.1 Polenta 374
17.4.2.2 Corn Flour as Raw Material for Gluten-Free Pasta 376
17.5 Typical and New Italian Products from Buckwheat 376
References 381

17.1 INTRODUCTION

Foods derived from cereals, especially wheat, have always played a significant role in the diet of the peoples of the Mediterranean area (Ferro-Luzzi and Branca 1995), thanks to the optimal environment for the cultivation of grains. The reason for the success and widespread diffusion of the so-called "Mediterranean diet" lies not only in its renowned nutritional characteristics, with its foods rich in complex carbohydrates such as starch (Willett and others 1995; Riccardi and others 2003; Biesalski 2004; Trichopoulou and others 2005), but also in the broad "technological versatility" distinctive to wheat.

After being ground and mixed with water, this raw material is transformed into a cohesive dough that can be molded into different shapes (Serventi and Sabban 2000) to produce foods with highly diversified textures. This is possible, thanks to the exceptional viscoelastic properties of gluten (Hoseney 1989).

From wheat we can therefore obtain foods such as pasta. Pasta is a processed food that has an unusual long shelf-life before being cooked, thanks to its low moisture content and high compact texture. Its macromolecules have exceptional hydrating capacity, which enables it to increase its weight two-fold and acquire a palatable structure when cooked in boiling water. The pasta can then be combined with many different kinds of sauces to suit every taste (Antognelli 1980). Although pasta is doubtless the Italian food "par excellence", it would seem that the Greeks and the Romans were not familiar with this product in its present form (Serventi and Sabban 2000). We cannot find any explicit reference to pasta in any cooking texts until the end of the Middle Ages. Other sources from the twelfth century testify to the diffusion of this product from Italy and throughout the Mediterranean basin.

Wheat flour can also be transformed into other foods that have different physical properties from pasta, such as bread and all so-called baked products. These are characterized by a particular physical structure with a highly fragrant crust that encloses the internal alveolar-textured crumb that is often soft and light. Tasting and chewing a food with these properties produces a strong sense of pleasure and fully satisfies all our sensorial "requirements", from sight to touch, and from smell to taste. For these reasons, the social and religious importance given to bread by peoples of the Mediterranean throughout history is fully justified. Inscriptions of the ancient Rome era include bread in the list of indispensable foods for the people of Rome, administered by the Annona, the administrative organization that provided the city with victuals (Rebora 2000).

Because of Italy's specific configuration, it forms a narrow peninsula approximately 1200 km long, with 75% of the area being covered by hills and mountains. The cultivation of common wheat is mainly limited to the plains of central-northern regions, where it is in

competition with corn, a cereal that assures a higher productivity. The pedo-climatic characteristics of the Alpine and Apennine regions, where intensive farming is not possible, have given rise to the development of other crops (cereals and noncereals), able to assure fairly good productivity and therefore being economically profitable. This explains the significant diversification of raw materials used for bread-making and other cereal products in Italy, a tradition that has found renewed interest with both consumers and the scientific community in recent years.

In particular, the resistance of rye to the harsh winter temperatures of the Alps and its greater availability in this area justify the large number of types of rye-based breads produced over the centuries throughout the entire Alpine area and which are still produced and consumed there (INSOR 2000). Sourdough leavening is also preferred, given the low bread-making quality of rye, in accordance with the traditions of the central and north European countries where this cereal is widely used for bread-making (Katina 2003; Stolz 2003). On the other hand, breads containing emmer wheat (*Triticum spelta*), barley, potato, and chestnut flours are still common in central Italy (Bordo and Surrasca 2002), an area with a milder climate but with a soil that is not suitable to intensive farming. Agronomic conditions in southern Italy (Puglia in particular) and in the two main islands Sicily and Sardinia, where durum wheat (*Triticum durum* Desf.) has been cultivated for thousands of years with excellent results in both quantity and quality, are completely different. The most frequent types of breads produced, consumed, and appreciated in these areas contain only re-milled semolina of durum wheat (semolina with a fine particle size). They are made using the sourdough process, a technology that achieves better results with this particular raw material (INSOR 2000; Consorzio Gian Pietro Ballatore 2001; Bordo and Surrasca 2002).

In this chapter, we will discuss the production processes and characteristics of the most well-known starch-based foods of Italian tradition, with special emphasis on the evolution of the artisanal processes, which in many cases have led to an optimization and transformation of their production into a truly industrial cycle.

Another important aspect closely connected to the reason for the widespread popularity of both dry and fresh pasta and baked products throughout Italy involves machines. A large number of manufacturers have made available to the artisans and industrial sectors high-quality equipment to make pasta and bread. This segment holds a strategic position in Italian manufacturing. The trade directory for the sector (Leone 2004) states that the production of machinery for the bread- and pasta-making industry represents more than 50% of the total production of installations for the food industry. The expertise of Italian manufacturers in this field is also demonstrated by the significant sales turnover for cereal transformation machinery, totaling about 53% in 2002.

17.2 PASTA

17.2.1 A Brief History

There are very few documents attesting to the true origins of pasta and this has given rise to numerous legends. Some attribute the origins of pasta to Italy, and others to more distant lands, perhaps even China (Serventi and Sabban 2000). A product similar to the current *lasagna* was known to both the ancient Romans and to the Etruscans (Agnesi 1996). However, it is not until the twelfth century that can we find references to and documents about this food. In the early twelfth century, the Arab geographer Al-Idris reported the existence of the production of a dried pasta in Sicily, which was distributed throughout the Mediterranean basis, thanks to its long-term preservation properties. Its production was strictly a

"family" business, and it was not until the mid-1300s that we have documented evidence of the first artisan pasta shops. Despite its Sicilian origins, the pasta-making industry expanded and flourished in Naples (Portesi 1957; Serventi and Sabban 2000).

Together with the production of dried pasta, an activity that was mainly concentrated in southern Italy and the Ligurian coasts, thanks to a microclimate that facilitates the drying of the product, we also have documented evidence of small pasta-making businesses that supplied fresh pasta to local demand. These artisans were active in central-northern Italy, where durum wheat semolina was more difficult to find. This explains why the majority of fresh pasta is still today produced with common wheat flour, often with the addition of eggs, an important ingredient both from the point of view of nutrition and technology. The *vermicellai* (pasta-makers) gave way to factories in the early 1900s, when the first electric motors appeared. Surely the introduction of the continuous press for the kneading process around the 1930s represented a decisive innovation for the development of this product. The production process, which had been discontinuous until this time, became continuous, giving rise to greater productivity and greater cost-savings (Portesi 1957; Serventi and Sabban 2000).

17.2.2 Dried Pasta

The reasons for the success and diffusion of pasta worldwide in the last few decades include the possibility of using other kinds of cereals in its production, not just durum wheat (UNIPI 2006). According to the producing country and its culinary traditions, other cheaper and more easily available cereals can be used. In any case, the ingredients and technological processes required for pasta production are extremely simple (Pagani 1986; Lucisano and Pagani 1997). Today, pasta is a product that well represents "globalization", because it comprises the traditional and typically Italian dried pasta made of semolina, with or without eggs, as well as the oriental pasta made of rice or leguminous flour (Pavan 1998).

As shown in Figure 17.1, and in accordance with the provisions of Italian Legislation (1967 and amendments), pasta-making involves the kneading of the semolina with water. The mass is then worked into various shapes using two technological methods, extrusion under pressure and/or lamination, and is finally dried and packaged. These operations ensure a three-year preservation of the dried pasta and, given the absence of any additives or preservatives, it is a truly natural product.

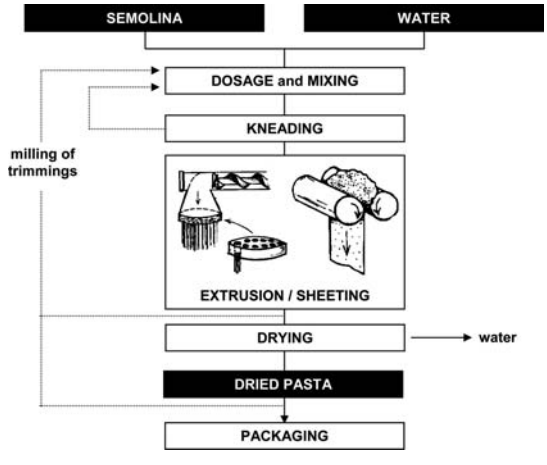


Figure 17.1 *The pasta-making process.*

TABLE 17.1 Italian Production of Pasta[a].

		Italian Consumption		
	Total Production (t)	Total (t)	Percapita/Year (kg)	Total Export (t)
Dried Pasta	3,023,598	1,493,540	26	1,530,058
Semolina pasta	2,735,800	1,386,400	–	1,349,400
Egg pasta	179,175	98,830	–	80,345
Stuffed pasta	108,613	8,310	–	100,313
Fresh Pasta	98,000	98,000	2	

[a]Data refer to 2004.

Other factors have increased interest in pasta during the last few years, especially overseas. Together with the traditional product, prepared exclusively with cereal flours, there are other similar products that are the same in shape but have a higher nutritional value because of the addition of different kinds of proteins (egg pasta, pasta enriched with whey proteins, and so on), vitamins, and mineral salts. These products can be dried as well as ready-to-use, that is, already cooked and with the sauce added (convenience foods). Finally, the rediscovery of the nutritional benefits of the so-called Mediterranean diet has finally rid this food of the stigma of being a "fattening food" (Ferro-Luzzi and Branca 1995).

17.2.2.1 Definition of the Product and Quality Characteristics. Dried pasta is without doubt the favorite kind of pasta in Italy. It represents approximately 90% of the purchasing volume of the sector (Table 17.1). Pasta therefore plays a significant part in the Italian diet, as demonstrated by the high yearly consumption, approximately 26 kg per capita/year and still slowly increasing (UNIPI 2006). Fresh pasta is also becoming more and more popular. According to Italian law (1967), the appellation "fresh pasta" refers to the moisture content of the food. This product will be discussed in detail in Section 17.2.3. Although this classification criterion based on "final moisture" is the one most commonly used (Fig. 17.2), there are other useful parameters for identifying the different kinds of pasta, such as ingredients (egg, vegetables, and so on), process, and consequently the type of format (long/short pasta, laminated/extruded pasta). The most suitable raw material used for the production of dried pasta, and the only one allowed by Italian law (as also in France and Greece), is

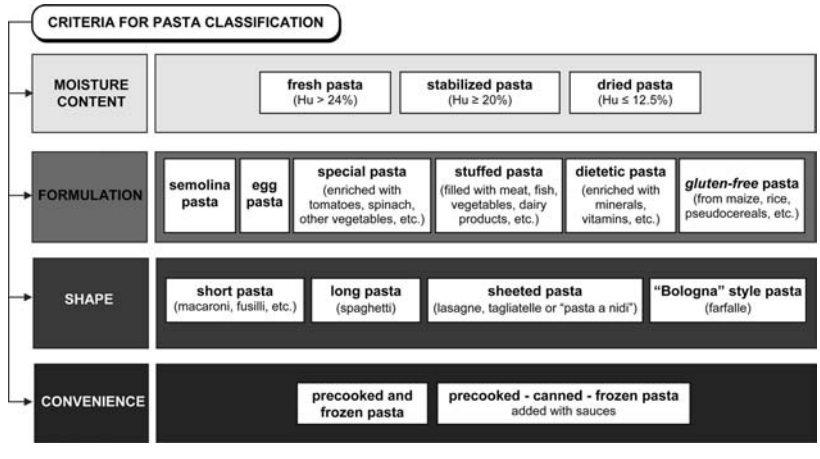


Figure 17.2 *Pasta classification.*

durum wheat semolina (Italian Legislation 1967). This raw material differs in many ways from common wheat flour, both from the point of view of the chemical parameters (as required by law, Table 17.2) and its rheological characteristics, useful for predicting the behavior of the raw material during processing (D'Egidio and others 1990; UNI 2001).

TABLE 17.2 Semolina and Wheat Flour Properties According to Italian Law and to Italian Voluntary Classification.

	Semolina Properties – Italian Law (L 580/1967 and Amendments)			
	Moisture (%)	Ash (%db)		Protein (%db)
From Durum Wheat	Max	Min	Max	Min
Semolina	14.50	–	0.90	10.50
Coarse semolina	14.50	0.90	1.35	11.50
Wholemeal semolina	14.50	1.40	1.80	11.50
Re-milled semolina	14.50	1.36	1.70	11.50

	Italian Voluntary Classification (UNI 10940, 2001)				
From Durum Wheat	Protein (%db)	Dried Gluten (%db)	Gluten Index	W ($\times 10^{-4}$ J)	P/L
Semolina "class A"	$\geq$13.5	$\geq$12.0	$\geq$80	$\geq$250	2.0–2.5
Semolina "class B"	12.0–13.5	10.5–12.0	60–80	180–250	1.0–2.0
Semolina "class C"	10.5–12.0	9.0–10.5	30–60	100–180	0.5–1.0

	Wheat Flour Properties – Italian Law (L 580/1967 and Amendments)			
	Moisture (%)	Ash (%db)		Protein (%db)
From Common Wheat	Max	Min	Max	Min
Wheat flour "type 00"	14.50	–	0.55	9.00
Wheat flour "type 0"	14.50	–	0.65	11.00
Wheat flour "type 1"	14.50	–	0.80	12.00
Wheat flour "type 2"	14.50	–	0.95	12.00
Wholemeal flour	14.50	1.30	1.70	12.00

	Italian Voluntary Classification (Source: Aldovrandi and Vitali, 1995)				
From Common Wheat	Protein (%db)	W ($\times 10^{-4}$ J)	P/L	Stability (%db)	FN (s)
Improver wheat (very strong gluten)	$\geq$14.5	$\geq$300	$\leq$1	$\geq$15	$\geq$250
Superior bread-making wheat	13.5–14.5	220–300	$\leq$0.6	10–15	$\geq$220
Ordinary bread-making wheat	11.5–13.5	160–200	$\leq$0.6	5–10	$\geq$220
Wheat for cookies (weak gluten)	$\leq$10.5	$\leq$115	$\leq$0.5	Not required	$\geq$240

%db: percentage dry basis; W and P/L: alveographic indices; stability: farinographic index; FN = falling number.

TABLE 17.3 Characteristics of Dried Pasta According to Italian Law and to the Requirements of Italian Consumers.

	Pasta Characteristics – Italian Law (L 580/1967 and Amendments)						
	Moisture (%)	Ash (%db)		Protein (%db)	Acidity[a]	Lipids (%ds)	Sterols (%db)
Raw Material	Max	Min	Max	Min	Max	Min	Min
Semolina	12.50	–	0.90	10.50	4	–	–
Coarse semolina	12.50	0.90	1.35	11.50	5	–	–
Wholemeal semolina	12.50	1.40	1.80	11.50	6	–	–
Semolina and eggs	12.50	–	1.10	12.50	–	2.80	0.145

%db: percentage dry basis.
[a]The amount of KOH 1 N (mL) necessary to neutralize 100 g of dried pasta.

Pasta Characteristics – Consumer Requirements	
Appearance	Cooking Quality
Typical yellow color	Optimum consistence
Black specks: absent	Lack of stickiness
White spots: absent	Lack of bulkiness
Fractures, fissures: absent	

In particular, the functional properties of proteins in the durum and the high pigment content contribute to a high-quality product.

However, the characteristics that pasta must have, to be judged to be of good quality by the consumer, often do not coincide with those required by law, and may be highly different according to consumer eating habits (Table 17.3). In other words, the evaluation scale is highly subjective, because it is based on personal preference (Cole 1991; D'Egidio and Nardi 1996). It is important to remember that the judgment of pasta does not consider the nutritional value of the product, a characteristic that can be greatly influenced by the technological process, as will be discussed later.

The consumer's final judgment will surely consider the appearance of the product in its dry state and its behavior when cooked. To this end, it is useful to note briefly the role and behavior of the two main components of semolina and pasta, that is, starch and protein. In dried pasta, the starch is still in the form of whole native granules, as in semolina. The proteins, arranged more or less uniformly and regularly between each granule, are mostly interactive and form the gluten complex (Dexter and Matsuo 1977; Resmini and Pagani 1983).

During cooking, the starch granules and proteins behave in a completely different manner. The starch granules swell up rapidly and tend to disperse and in part become soluble. The proteins, on the contrary, become completely insoluble and coagulate, creating a netlike structure (Fig. 17.3). Because these phenomena occur at approximately the same temperatures, the more rapidly the proteins form a compact netlike structure during cooking, the more limited will be the swelling of the starch. The starch components will remain trapped inside the protein network, ensuring a firm consistency and absence of stickiness (Resmini and Pagani 1983). However, if the netlike structure lacks elasticity or its formation is delayed, the starch granules are not confined and will swell up easily. Part of the gelatinized and soluble material will pass into the cooking water. The product will be judged sticky and of poor consistency. It is therefore easy to understand how good cooking quality depends on a high content of protein in the semolina and the proteins'

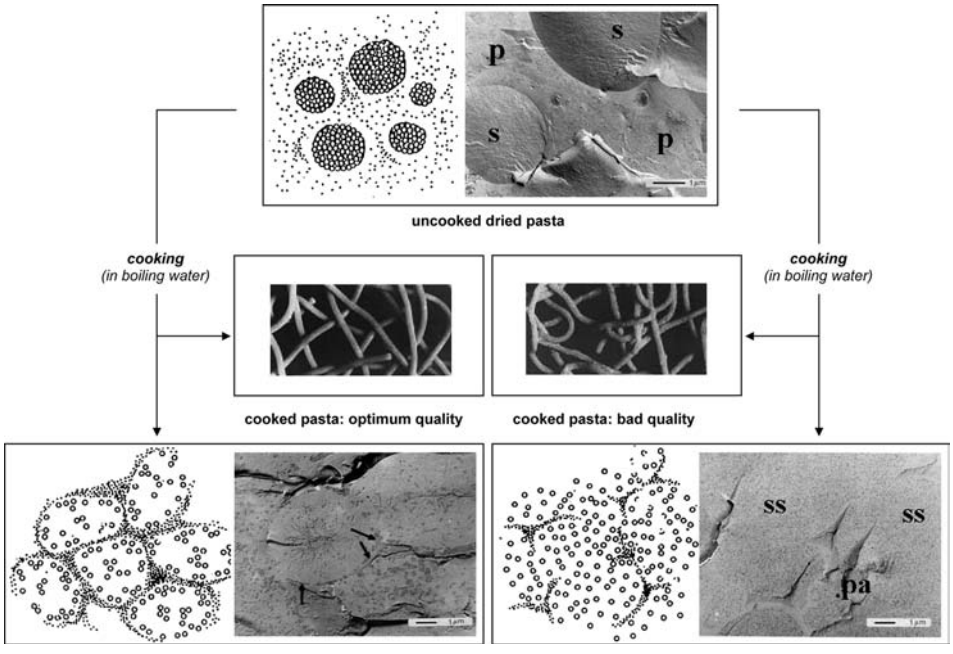


Figure 17.3 *Starch and protein organization in uncooked and cooked pasta (p = protein; s = starch; ss = swollen starch; pa = protein aggregates; arrows = protein network).*

readiness to reticulate and form gluten. A number of studies have been made to select new varieties that better satisfy the requirements of pasta producers.

17.2.2.2 Production Technology. As well as being influenced by the properties of the raw material, pasta quality is also influenced by the conditions during the pasta-making process. The effects on the components can be highly different, affecting the outcome of the competition between starch and proteins during cooking (Resmini and Pagani 1983). Although pasta is a food that has been known for centuries, its production process is still today undergoing technological innovation, more often than not dictated by production needs. For a detailed description of the process, the individual operations thereof and the characteristics of the finished product, see Kruger and others (1996) and Kill and Turnbull (2001).

The pasta-making process today is performed completely via automation (Fig. 17.4). The semolina is taken to the first section of the continuous press via a pneumatic system. Here it is kneaded with water to obtain a dough of approximately 30% moisture. During this phase, which lasts about 15–20 min in traditional machines, it is important to ensure a uniform moisture content of the semolina particles to prevent white spots in the finished product.

The moistened mass has the appearance of irregularly shaped lumps and is extruded in the second part of the press, which comprises a horizontal cylinder with a worm screw that ensures the movement of the dough and facilitates the interaction of the hydrated proteins and the formation of gluten. This operation is mainly carried out in vacuum to prevent loss of shine and attenuation of color caused by the intrusion of tiny air bubbles and the oxidation of the semolina natural pigments (Feillet and Dexter 1996). The compression screw then pushes the dough towards the die, a bronze plate (a material capable of resisting the pressures inside the machine), with different holes according to the shapes desired, and usually lined with Teflon to obtain a smooth surface of the product.

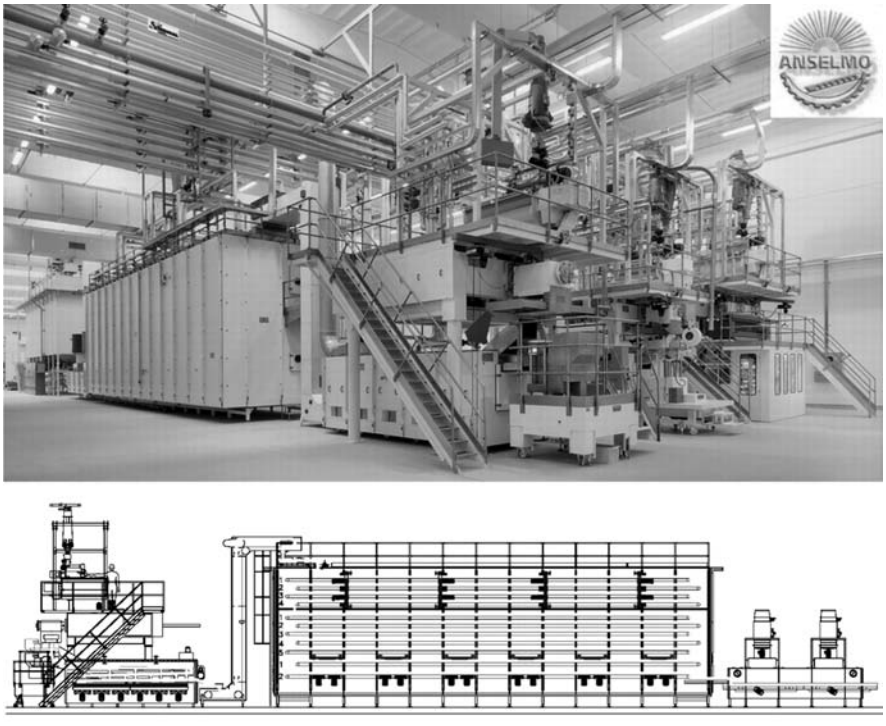


Figure 17.4 *Automated pasta-making set-up. (Courtesy of ANSELMO, Italy).*

If a bronze die is used, this gives a rough surface, which, according to some consumers, is preferable as it enables the pasta to hold the sauce better. Significant pressure develops inside the machine during this phase of the process, called extrusion under pressure, which can even exceed 10 MPa (~100 atm) (Pagani and others 1989; Dawe and others 2001). The low fluidity of the mass also produces high friction with increases in temperature. This is an undesirable effect, which can be controlled in part via the cylinder water jacket, which assures a temperature of 40–45°C. Higher temperatures would stimulate the swelling of the starch granules and coagulation of the proteins into irregular masses. Both these phenomena would have negative effects on the pasta behavior during cooking. The present equipment control systems prevent damage to the product. The pasta that is expelled from the die reflects the quality characteristics of the type of semolina/flour used (Pagani and others 1989; Dalbon and others 1996).

The innovations, even the most recent, regarding this first phase of the pasta-making process that have evolved over the years have focused on optimization of machine performance from an engineering point of view, but have not substantially changed the way the dough is shaped. All the main producers for pasta-making equipment have dedicated special attention to the elimination of one of the most frequent defects of dried pasta, the "white spots" formed due to the presence of unmoistened particles during the prekneading phases, which are clearly noticeable in the finished product. Not only does this defect have a significant effect on the hesthetic appearance of the product, but when observed through a microscope, it shows an incomplete and nonhomogenous structure of the protein network structure, which results in a high fragility of the pasta both in its dry state and during cooking. The appearance of white spots can be reduced by using fine-grain semolina

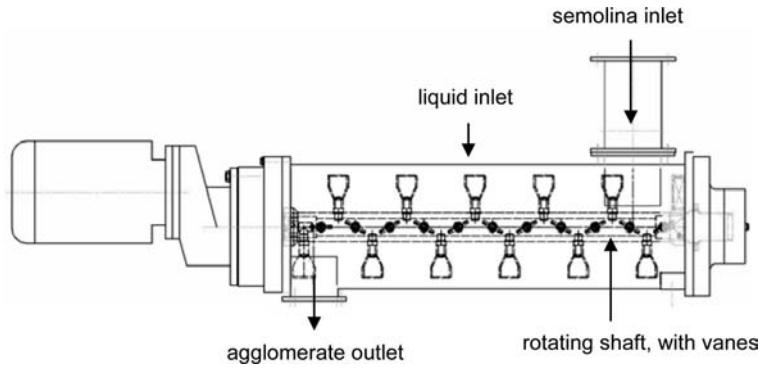


Figure 17.5 *The "centrifuge" – a high speed horizontal premixer. (Courtesy of PREFOR, Italy).*

(with a particle diameter of less than 300–350 μm) (Dalbon and others 1996). However, some pasta producers consider this type of semolina to be of poorer quality due to the mechanical stress induced on the starch and proteins during milling (Pagani and others 1996).

The most frequently adopted technological configuration is the so-called "centrifuge", a high-speed horizontal premixer (Fig. 17.5). The function of this machine is to distribute water between the semolina particles in the most homogenous way possible. More recently, a number of equipment manufacturers have contributed to the optimization of the mixing phases and of the subsequent kneading with interesting and highly diverse technological configurations. The extrusion press, with co-rotating screws, developed by Bühler a few years ago (Dawe and others 2001), improves both the productivity and hygiene aspects of the process (Fig. 17.6). Another alternative is the "dough stabilization belt" proposed by an Italian company for pasta equipment (Fig. 17.7), a conveyor belt that collects the dough and conveys it to the vacuum mixer, and then to the press. This configuration assures considerable ease of cleaning in this part of the process and a progressive moistening of the semolina without mechanical stress. Furthermore, the formation of a layer of ~15–20 cm of dough limits the quantity of oxygen that comes into contact with the mass, preventing the oxidation of most of the carotenoid pigments and avoiding discoloration.

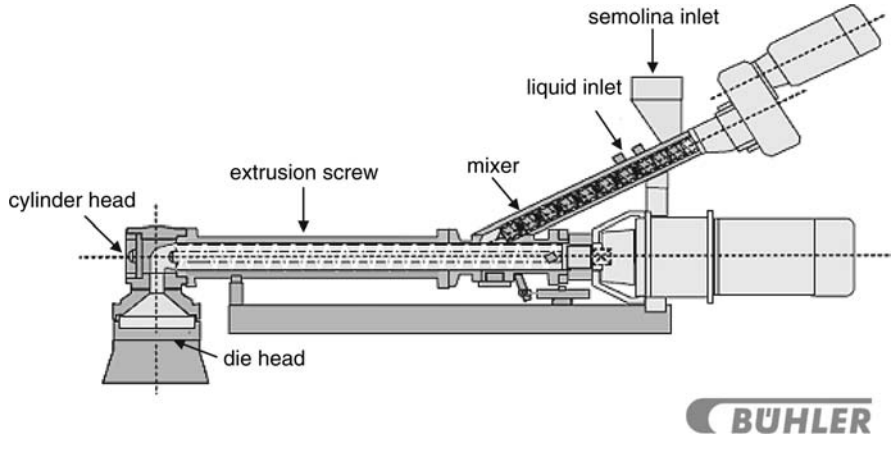


Figure 17.6 *The extrusion press with co-rotating screws – "Polymatic®". (Courtesy of Bühler, Switzerland).*

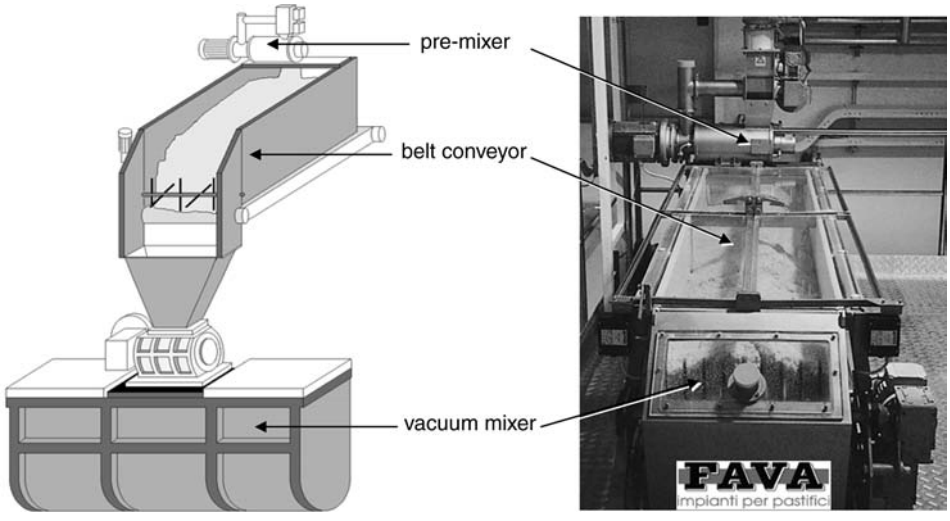


Figure 17.7 *"The dough stabilization belt". (Courtesy of FAVA, Italy).*

Another system for obtaining liquid–solid agglomerates with maximum uniformity is provided by a special appliance called the Vortex® or "vertical imbibition device". The machine works on aerodynamic principles and is essentially divided into three parts (Fig. 17.8): a vertical cylindrical chamber, a rotating shaft with a series of blades, and

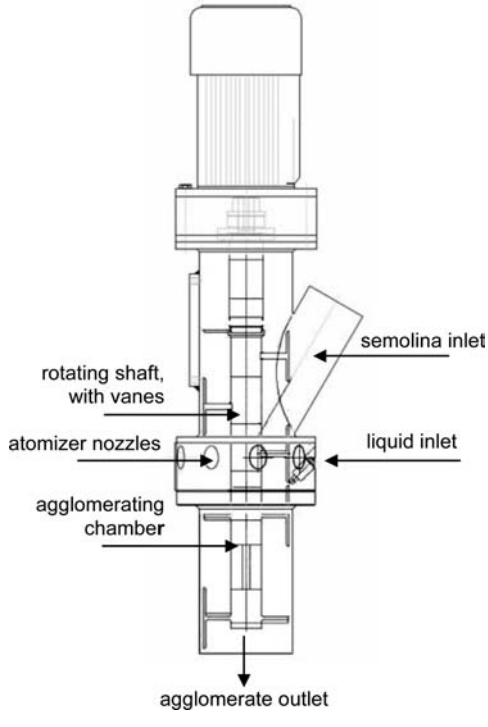


Figure 17.8 *The Vortex® device. (Courtesy of PREFOR, Italy).*

spray nozzles for the distribution of water. An electric motor drives the shaft, which is situated vertically inside the imbibition chamber. Thanks to their rotation speed and insertion angle in the shaft, the two blades produce a vortex. Each single semolina particle "floats" in the imbibition chamber and then falls into the mixer machine only after having been duly moistened, which is possible only after having reached a "threshold" specific weight.

Upon extrusion, the fresh pasta has a moisture content of about 30%, with the resulting low preservation characteristics at room temperature. Drying has the objective of lowering the moisture content down to 12.5%, the maximum value set by our laws and which ensures a water activity (a_w) of less than 0.6 (room temperature), enough to prevent the undesired proliferation of microorganisms and to reduce the numerous enzymatic activities. At the same time, the product acquires its characteristic flavor, aroma, and consistency. The drying process has always been, and still is today, the most crucial phase of the entire pasta-making process. A clear indication of this is the surface area required for the drying chamber, the time required for the operation (which varies according to the shape of the pasta; even the shortest cycle requires several hours) and the innovations dedicated to this phase presented by pasta equipment companies.

Drying the pasta does not only mean evaporating the water. This operation must be performed according to the chemical-physical structure of the product. Above all, stress that could occur in the different areas and between the pasta components due to the nonhomogenous distribution of water between the surface and internal areas and due to the different affinities of proteins and starch with water must be avoided. The moisture and volume of the pasta must be reduced uniformly and homogenously (Antognelli 1980; Paraventi 1984).

Until the last century, successful drying depended solely on the ability of the pasta-maker and the existence of specific environmental–climatic conditions such as those of the areas overlooking the gulf of Naples and the gulf of Genoa (Portesi 1957; Serventi and Sabban 2000). The drying cycle of long pasta could last as long as several days and required a considerable number of workers (Lirici 1983).

The technological progress that took place at the beginning of the twentieth century finally freed the drying phase from being dependant on climatic conditions. From the 1940s onwards, this phase was carried out in a tunnel with an artificial climate with air at controlled temperature and moisture, thus controlling the kinetics of the drying process.

The pasta drying cycle can, for the sake of simplicity, be divided into phases. The first stage is called the pre-drying phase (in Italian, *incartamento*, from the word *carta*, paper, due to the fine surface crust that is formed). During this phase, the pasta can lose water very quickly, up to a third of that added during kneading.

Upon completion of the pre-drying phase, the moisture present in the pasta, ($\sim$18–20%) varies in distribution between the surface (up to 15% moisture) and the internal part, where the water content is comparable to the state of the pasta upon extrusion. To prevent cracking (*bottature*) of the pasta caused by physical stress between the areas of different water content, it is vital that the drying cycle has so-called "resting" periods in high-humidity environments, in order to enable a homogeneous distribution of moisture inside the product (Paraventi 1984). Until the end of the 1980s, the drying cycle included periods of drying or "ventilation", alternated with periods of rest, determined by selecting the appropriate hygrometric conditions inside the dryers. Today's machines are designed with only one water evaporation phase, followed by a long stabilization phase for obtaining a uniform distribution of the moisture. At the end of the drying cycle, the pasta is cooled at ambient temperature before being packaged.

Until the 1970s, the maximum temperatures used for the drying cycle were 50–55°C (today defined as "low temperatures", or LT). Under these conditions, over 24 h were necessary to dry the long-shaped pasta. Fermentation could thus occur, and some kinds of fermentation could also be desirable as they gave the product characteristic tastes and aromas. However, no significant changes to the functional properties of the semolina components were noted (Resmini and Pagani 1983).

Quality during cooking was strictly linked to the quality of the raw material used. Making pasta with excellent-quality semolina was, in those days, the only guarantee for obtaining a product with good cooking properties.

The technological innovations in this scenario were obviously based on the search for greater cost saving, together with greater hygiene and constant quality of the product. One of the most logical and technologically easiest solutions was to raise the temperature up to 70–90°C and over ("high-temperature" diagrams, or HT) (Manser 1979). These conditions provided a drastic reduction in the processing time, up to 10-fold (4–8 h according to the pasta shapes), with consequently significant advantages from the points of view of cost, hygiene, and appearance (the dried pasta had a deeper color). The improvements in the quality level of the product observed during cooking were unexpected and even surprising at times when considering the characteristics of the raw material. These changes can be attributed to the changes induced by the HT treatment on the starch and above all on the proteins (Dexter and others 1981). In suitable temperature and moisture conditions (from 70°C with a sample moisture of less than 16–18%), proteins in the raw product coagulate and create a structure very similar to the one formed during cooking. This kind of network is effective in preventing an excessive swelling of the starch granules, thus assuring low stickiness and high firmness (in Italian, *nervo*) in the cooked pasta (Resmini and Pagani 1983).

The changes attributable to the HT drying diagrams are not, however, all positive. These conditions have a considerable influence on a number of sensory properties, resulting in a dullness of taste, excessive browning, and, above all, high thermal damage in the dried pasta (as discussed in Section 17.2.4).

17.2.3 Fresh Pasta

Although fresh pasta is used throughout Italy on special occasions, it is particularly popular in the northern-central regions, where dried semolina pasta was historically less known and consumed (Serventi and Sabban 2000).

In Italy, the market for fresh pasta is much smaller compared with dried pasta, but demand for both industrial and artisanal products is continually growing (Fig. 17.9). One of the reasons for the success of this segment is the consumer's subconscious belief in the close relationship between "freshness" and artisanal production, together with the opinion that artisanal production is the best expression of "mild technology". It should be stressed, however, that industrially produced fresh pasta is often obtained using highly sophisticated technological processes (a detailed description is given in Sections 17.2.3.4 and 17.2.3.5) in order to guarantee product freshness and authenticity, and at the same time ensuring the prolonged storage time and "convenience properties" demanded by the consumer.

In contrast with the marked commercial expansion of this sector, the scientific world gives little attention to the field of fresh pasta. If we consider the period between 1990 and 2005, over 230 works were published on dried pasta worldwide (as cited by *Food*

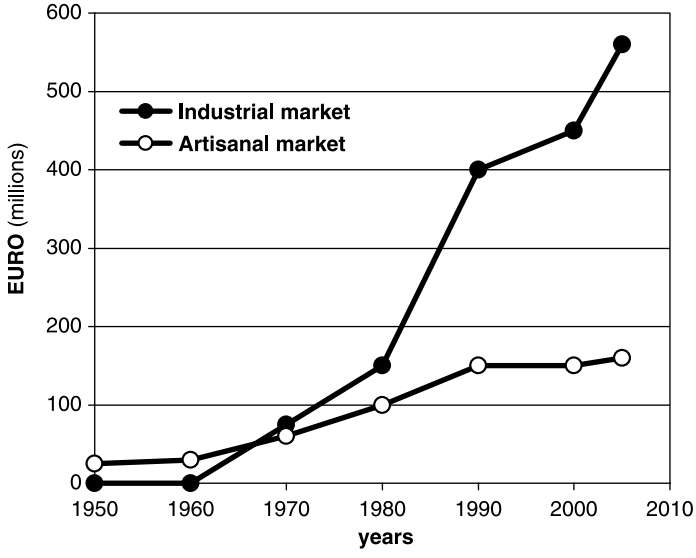


Figure 17.9 *Markets for industrial and artisanal pasta between 1950 and 2005.*

Science & Technology Abstracts). These studies were made to define the optimal characteristics of semolina, changes introduced to the process, in particular to the drying phase, and their influence on cooking behavior and nutritional properties. Only 84 works were published on fresh pasta during that same period, and most referred to the safety and hygiene problems involved and the new modified atmosphere packaging (MAP) technologies. Little attention was given to the influence of the raw materials and the process conditions on the properties of the finished product.

This chapter does not discuss pre-cooked pasta, as it has little economic relevance in Italy.

17.2.3.1 Classification Criteria. The classification criteria applied instinctively by the consumer to distinguish between "fresh pasta" and "dried pasta" correspond only in part to those applied by Italian law (1967; DPR 2001). As illustrated in Table 17.4, the

TABLE 17.4 Characteristics of Fresh Pasta According to Italian Law.

	Fresh Pasta Characteristics (Different Types, Having the Same Properties of Those Reported in Table 17.3, Except for:)					
Fresh Pasta	Moisture (%) Min	Acidity[a] Max	a_w Range	Heat Treatment (yes/no)	T Conservation (°C) Max (Tolerance)	Shelf-Life (days) Max[c]
Loose fresh pasta	24	7	—	No	4([b])	5
Packaged fresh pasta	24	7	0.92–0.97	Yes (pasteurization)	4 (±2)	60
Stabilized pasta	20	7	<0.92	Yes	Room temperature	30–120

[a]The amount of KOH in (mL) necessary to neutralize 100 g of dried pasta; [b]±3 during transport; ±2 in the other cases; [c]recommended.

law identifies the two categories on the basis of humidity, the value of which should not exceed 12.5% in dried products and should not be less than 24%, with a_w between 0.92 and 0.97, in fresh pasta. A third category, "stabilized pasta" is also described. This product must present a moisture content higher than 20% with a_w lower than 0.92. These properties are obtained by using specific thermal treatments and process conditions suitable to guarantee product transport and storage without refrigeration.

There are many different types of fresh pasta on the market (Fig. 17.2), but the ingredients in the basic formulation are simply flour and/or semolina and water. This basic recipe is often enriched with the addition of eggs. By law, there should be a minimum of four eggs per kilogram of flour. The richest and most complex formulations are those for the so-called "filled" or "stuffed pasta", where the pasta forms a shell that contains the filling or *farcia*. The filling is a soft mixture of animal and/or vegetable origin (meat, charcuterie, fish, cheese, vegetables, mushrooms, and so on), according to regional recipes and customs and availability. These ingredients are usually mixed with eggs and grated bread and comply with the health and authenticity requirements of the law (Italian Legislation, 1985).

Taking the shaping process as a classification criterion, we have the following:

1. "Long-cut pasta" or laminated pasta, made with a sheet of pasta about 1 mm thick, which is then cut into "ribbons" of different widths (for *lasagne*, *tagliatelle*, or *fettuccine*);
2. Short pasta with particular shapes (*orecchiette*, *trofie*, and so on); and
3. Products obtained by die-shaping (*fusilli*, *macaroni*, and so on).

In the cases of both long pasta and filled pasta, the formats vary in size and geometrical shape (Fig. 17.10) according to its consumption: "*pasta asciutta*" (pasta with sauce) or "*pasta in brodo*" (pasta in soup). Especially appreciated and renowned internationally are *tortellini* and *ravioli*, typical dishes of the cuisine of the Emilia region.

In the past, and even today, in artisanal products, the dough sheet represents a significant percentage of the product global gross weight, making it ideal as a single dish. The changes in eating habits and the need for faster cooking times has led to a preference for filled pasta where the pasta sheet is 0.5-mm-thick paper-thin (*sfoglia-velo*). This does not cover the flavor of the filling and requires just a few minutes' cooking. In this type of fresh filled pasta, the ratio of filling to dough is at least 50:100 (Table 17.5).

One of the most common criteria for classifying fresh pasta (apart from filled and unfilled) is its shelf-life (Fig. 17.2). The typical artisanal product is sold unpackaged and can be stored for a maximum of just five days, due to the absence of stabilizers. The commercial products have a longer shelf-life, from 30–40 days up to 120 days (Italian Legislation, DPR 2001). Given the high a_w values of the fresh product, this result can be achieved by applying pasteurizing heat treatments. The fresh pasta is then isolated from the environment in sealed packages where oxygen is removed using nitrogen and/or carbon dioxide atmospheres (MAP) and stored at low temperatures (+4°C).

These conditions (described in detail in Section 17.2.3.5) guarantee the hygiene of the product, a highly critical aspect, especially if the formulation contains eggs (usually included due to the positive effects at the technological level), and other animal and/or vegetable products used in special and filled pasta. These raw materials can often carry pathogenic microorganisms such as *Salmonella* and *Staphylococci*.

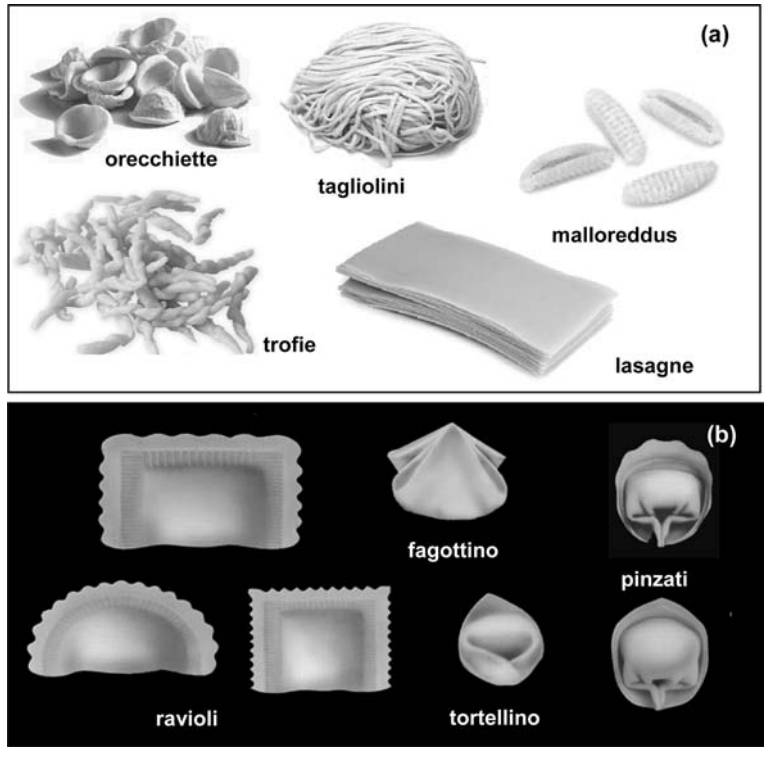


Figure 17.10 *Forms of pasta: (a) long and short pasta and (b) filled pasta.*

17.2.3.2 Quality Indices. Regardless of the technological process used, the fresh pasta produced in Italy must comply with the limits of microbial count set by Italian Legislation (1985) as illustrated in Table 17.6. These properties must therefore be considered as prerequisites for quality.

With regard to the characteristics considered by consumers, some may refer to the raw product, others to the behavior during cooking, as illustrated in Table 17.7 Anon. 2003). Before cooking, the laminated pasta must not contain any bubbles or fissures or tears, which are defects resulting from the kneading or rolling out and shaping of the dough. The color should be yellow, and the pasta must maintain its color even after cooking. Color can be improved by adding eggs and using flour and/or semolina with low enzymatic activities (lipoxygenase and oxidases). And lastly, as for dry pasta, good fresh

TABLE 17.5 Different Properties of Different Types of Artisanal and Industrial Stuffed Fresh Pasta.

Fresh Stuffed Pasta	Total Moisture (%)	Pasta Moisture (%)	Stuff Moisture (%)	% Stuff Weight (g/100 g mb)
Industrial *tortellini*	37.4	27.8 ± 0.8	47.6 ± 1.6	50 ± 0.10
Industrial *ravioli*	54.5	35.1 ± 3.8	69.8 ± 0.7	54 ± 0.03
Artisanal *tortellini*	22.4	24.4 ± 3.2	33.5 ± 3.3	32 ± 0.01
Artisanal *ravioli*	23.1	24.1 ± 0.4	34.4 ± 3.5	33 ± 0.04

mb: Moisture basis.

TABLE 17.6 Microbial Characteristics of Fresh Pasta.[a]

Raw Materials Used for Dough Preparation	Microbial Reference Value
Semolina	
Total aerobic count	30–40,000 cfu/g, max
Yeasts	500 cfu/g, max
Moulds	500 cfu/g, max
Salmonella	Absent in 25 g
Staphilococci	100 cfu/g, max
Enterobacteria	100 cfu/g, max
Fecal coliforms	100 cfu/g, max
Bacillus cereus	Absent
Water	
Total aerobic count	<150 cfu
Fecal streptococci	Absent in 100 mL
Fecal coliforms	Absent in 100 mL
Sporogenic sulfite reductive clostridia	Absent in 100 mL
Staphylococcus aureus	Absent in 100 mL
Pseudomonas aeruginosa	<100 cfu/100 mL
Pasteurized Liquid Egg	
Total aerobic count	10,000 cfu/g, max
Yeasts	1000 cfu/g, max
Salmonella	Absent in 25 g
Potentially pathogenic staphylococci	Absent in 25 g
Enterobacteria	<10 cfu/g, max
Bacillus cereus	Absent in 25 g
Product Before and After Heat Treatment	**Recorded Microbial Values**
Dough	
Total aerobic count	$<10^6$ cfu/g
Coliforms	<1000 cfu/g
Fecal coliforms	<100 cfu/g
Escherichia coli	<1000 cfu/g
Staphylococcus aureus	Absent in 25 g
Salmonella	Absent in 25 g
Pasta After Pasteurization	
Total aerobic count	<10 cfu/g
Coliforms	<10 cfu/g
Fecal coliforms	<10 cfu/g
Escherichia coli	<10 cfu/g
Staphylococcus aureus	Absent in 25 g
Salmonella	Absent in 25 g

[a]Adapted from Italian legislation, 1985.

products must absorb a high quantity of water while limiting stickiness and loss of material into the cooking water.

17.2.3.3 Raw Materials. The differences between fresh pasta and dried pasta can be seen in other aspects of the product. As opposed to dried pasta, it is possible to use

TABLE 17.7 Quality Indices of Fresh Pasta According to Italian Consumers.

Pasta Properties Before Cooking	
Appearance	Absence of bubbles, fissures, streaks
	Opaque surface
Color	Yellow
	High brightness
Rheological properties	Resistance to breakage during working
	Absence of stickiness
Pasta Behavior During Cooking	
Cooking loss	Limited
Increase in weight	High and rapid absorption of water
Color	Maintenance of color
Rheological properties	Absence of stickness

common wheat flour for the preparation of fresh pasta (Italian Legislation 1967). This is justified if we take into account the regions of origin of fresh pasta (with unsuitable environmental conditions for the cultivation of durum wheat, as previously mentioned), and the technological lamination process, which is not suitable for excessively strong dough. However, as there are no legal provisions regarding the percentage ratio of common wheat and durum wheat flours used, there is a high heterogeneity between the ratios used, as discussed by Pagani and others (1999) and Alamprese and others (2005a). The picture is further complicated by the lack of knowledge regarding the optimal characteristics of flours and/or semolina for this transformation. Last, but not least, the role and technological behavior of the flours may be concealed by the fact that most products, both artisanal and industrial, contain eggs, which not only give pasta its color but also its binding and structural properties. Each producer therefore uses formulations based on his own experience, and not on any specific basic knowledge. Consequently, even if we just consider fresh egg pasta, the composition of the products available on the market can vary widely within each parameter provided for in the law (Fig. 17.11).

With regard to the influence of eggs, a recent work by Italian authors (Alamprese and others 2005b) shows that the use of fresh or pasteurized eggs does not cause any significant change to the rheological properties of the pasta and its cooking behavior.

17.2.3.4 Pasta-Making Process. If we consider the production process as a whole, we can see marked differences with respect to dried pasta (Fig. 17.12). The final drying process is obviously left out, and the kneading and shaping operations of the dough are obtained using different methods (Pavan 1990). In particular, the ingredients are often mixed with a kneading machine called *gramola* (Fig. 17.13), which is exactly the same machine as those used historically in the discontinuous pasta-making process (Portesi 1957). The kneading machine mixes the ingredients slowly, without causing friction or heating the dough. In more automated industrial processes, the kneading is made inside vats with a single rotating shaft for 15–20 min (Fig. 17.14). At the end of the kneading process, the dough is sent to the dough sheeter, where it is pressed into a compact sheet by a couple of smooth rolls. The next stage is the calibration stage, which comprises a sequence of steps using smooth calibrators adjusted to give the thickness desired and a smooth and velvety finish.

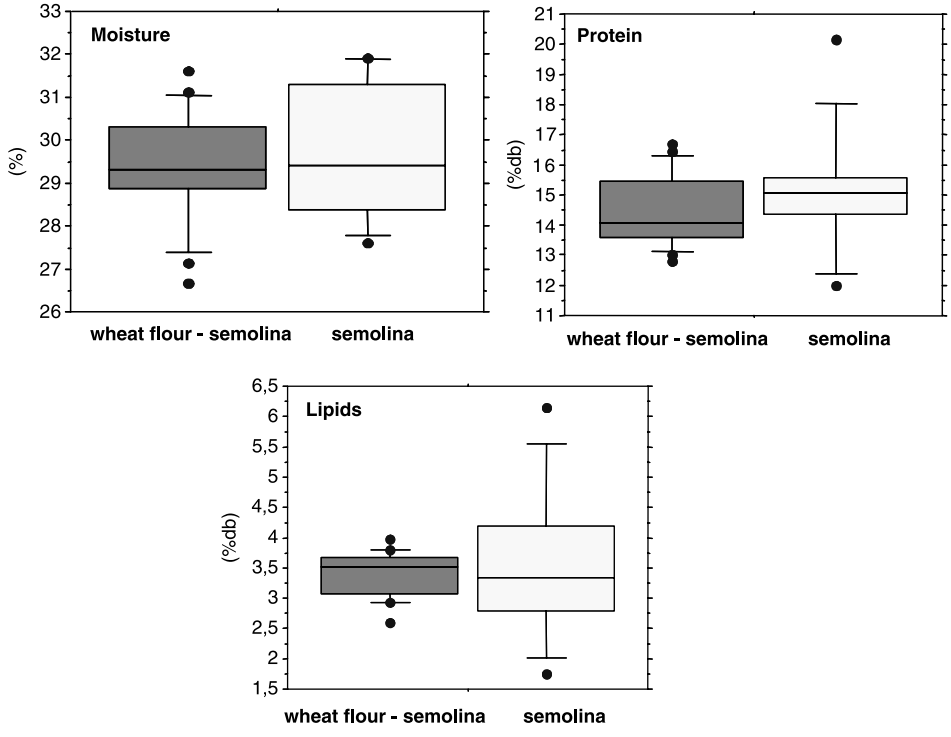


Figure 17.11 *Composition of pasta products with regard to moisture, protein, and lipids.*

The most recent innovations in the dough-kneading and sheeting phase relate to ease of cleaning and the possibility of reducing physical stress during dough lamination. A solution is given in Figure 17.15. This new machine, which is fully constructed in stainless steel, has three kneading rolls with a new self-cleaning design that eliminates dough accumulation and guarantees limited heating of the mass. A patented device allows dough mixing and kneading under vacuum, enhancing the yellow color and texture of the final product.

Industrial pasta sheets are usually given a final thickness of 0.5–1.5 mm. Some producers prefer to shape the first sheet (5–15 mm thickness) with an extruder. Although the extrusion head is such as to extrude the pasta at low speed, friction is formed, producing a rough surface on the product, a characteristic that is not eliminated in the successive calibration phases.

In the case of filled pasta, the filling is dosed by a machine that distributes it between the two sheets, producing *ravioli*, or on a single sheet that is and then folded and sealed to produce *tortellini*, *fagottini*, and so on.

Lamination with cylinders is a technology with a much lower productivity than extrusion under pressure, and, in general, makes it possible to obtain a better protein network of the gluten. The images obtained by the SEM in Figure 17.16 clearly show how the die-shaping process can cause heavy physical stress in the pasta, with a resulting breakage of the protein network when the raw material does not contain strong proteins, as in the case of common wheat. This three-dimensional organization justifies the modest performance in cooking, with the formation of large masses of gelatinized starchy material, which

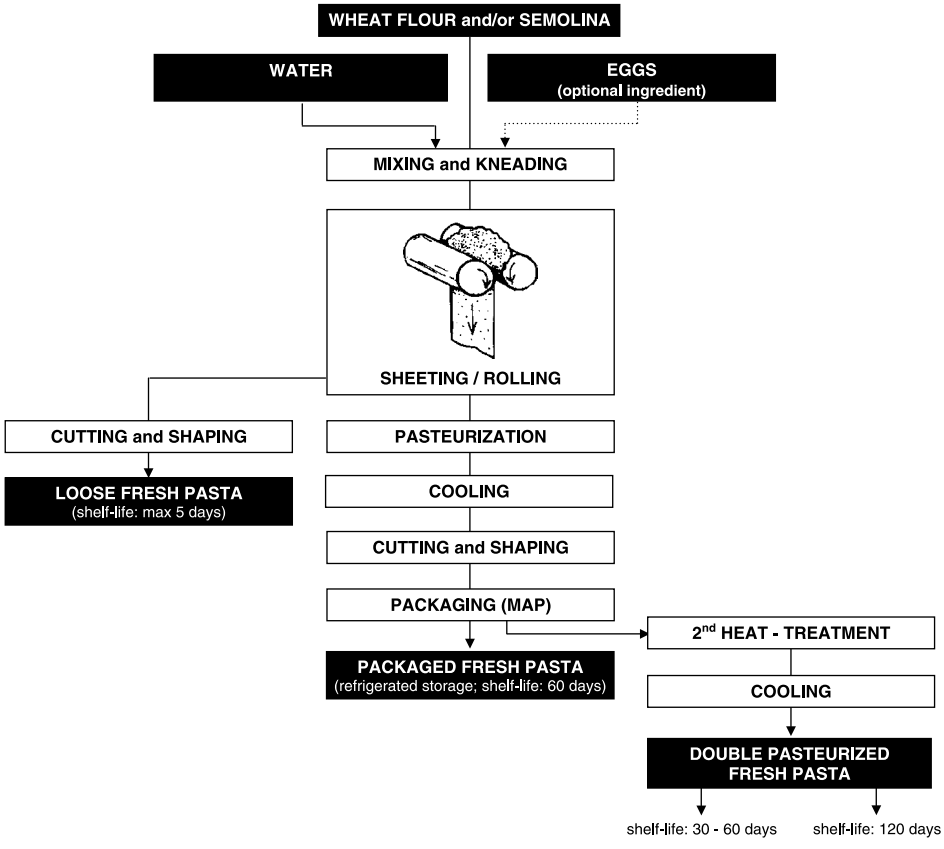


Figure 17.12 *Production process for fresh pasta.*

Figure 17.13 *Artisanal fresh pasta-making process: mixing (a); dough kneading with gramola (b); dough cutting (c); intermediate dough sheeting (d); final sheeting (e); pasta after the first pasteurization (f). (Courtesy of Fontaneto, Italy).*

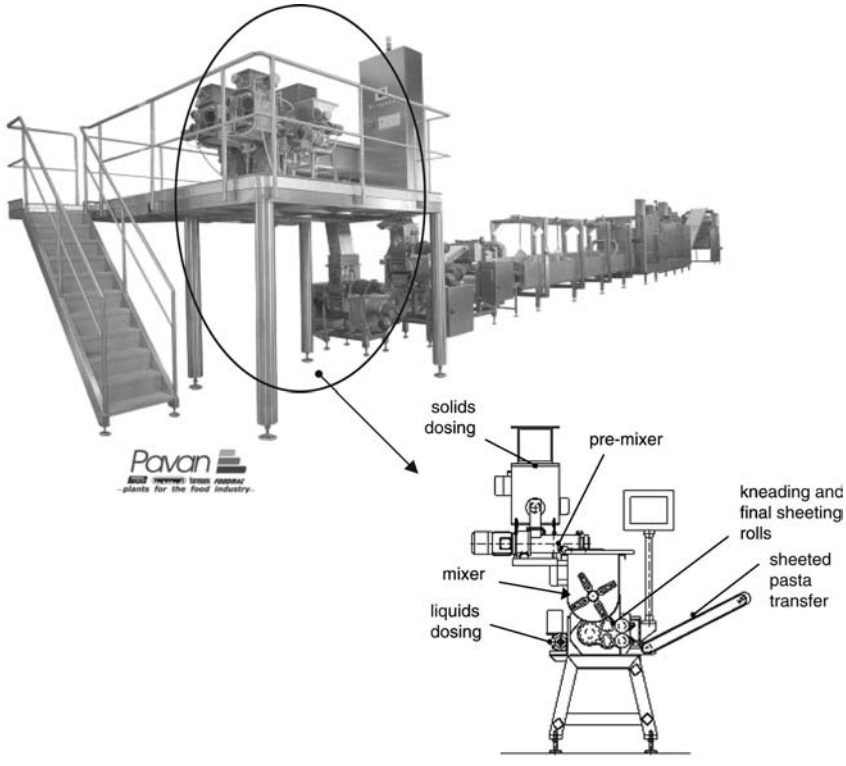


Figure 17.14 *Industrial-scale kneading machine for fresh pasta production. (Courtesy of PAVAN, Italy).*

is responsible for the starchiness of the water and lack of firmness of the pasta. However, from the same flour and with a sheeting process, it is possible to obtain fresh raw pasta with a well-developed and continuous protein network. In cooking, the fusion of the starch granules is limited by the presence of the protein network that surrounds each starch granules (Pagani and others 1989).

Whatever the production process used, fresh pasta is difficult to store and must be used within several days. A pasteurizing heat treatment must be applied to the product if we

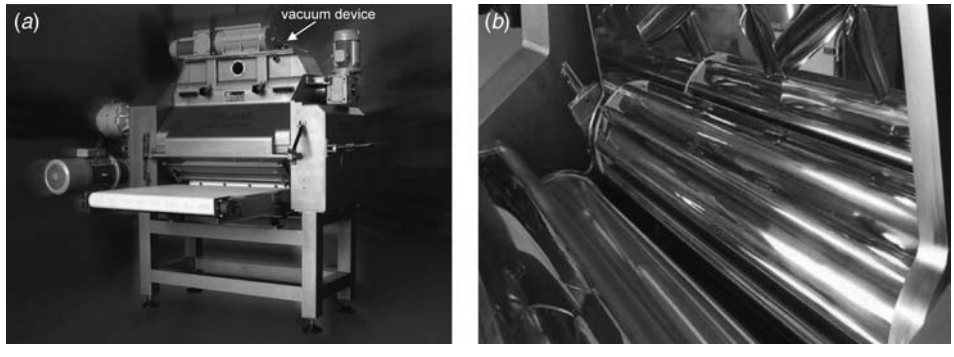


Figure 17.15 *Innovative dough-kneading machine (a) with self cleaning design (b). (Courtesy of STORCI, Italy).*

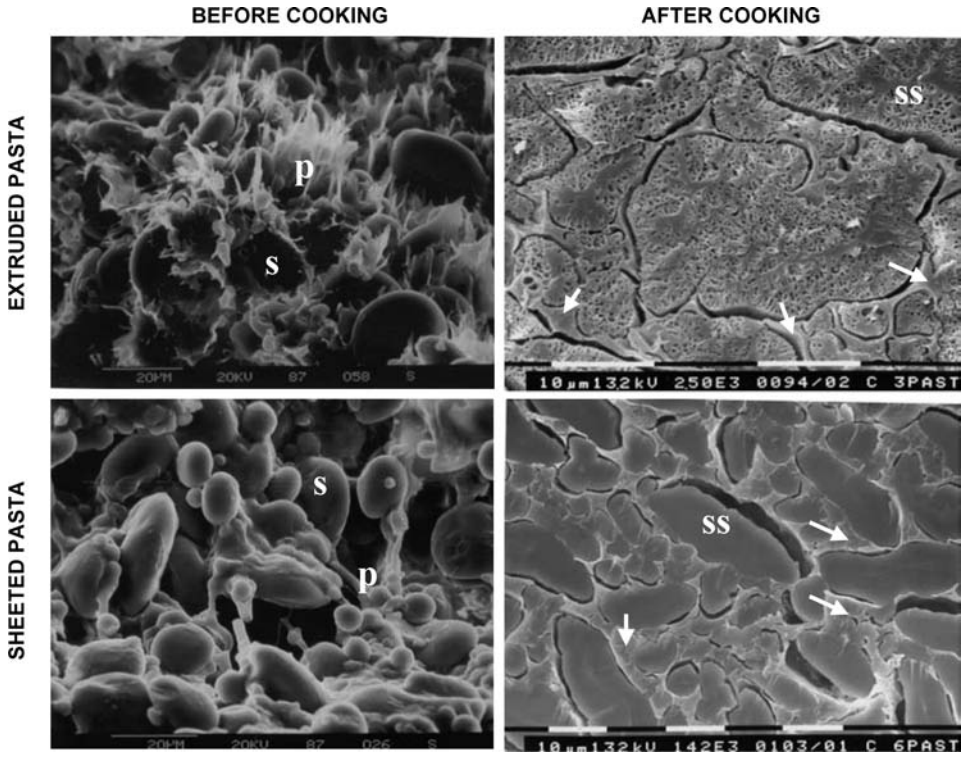


Figure 17.16 *SEM images of pasta after extrusion and sheeting processes (p = protein; s = starch; ss = swollen starch; arrows = protein network).*

want to prolong the shelf-life. However, this process must not compromise the nutritional and sensory characteristics, as stated by Mondelli (2003a,b). The conditions adopted are highly variable, due mainly to the number of stabilization heat treatments applied. Today, production processes with a single pasteurizing treatment are preferred. The conditions also vary according to the thickness of the pasta sheet and the filling (whose ingredients contribute to the potential presence of microorganisms), and the proportion of the amount of filling to the amount of pasta sheet.

The fresh product is loaded onto a conveyor belt that enters the pasteurization tunnel where steam is injected from the opposite end (Fig. 17.17). Treatment with saturated steam, however, produces condensation on the surface of the pasta. This layer of water could induce starch gelatinization, causing pieces to stick together and giving the pasta a shiny surface (Mondelli 2003a). These defects can be avoided by using superheated steam, although this technology is more costly.

The most frequently used conditions for pasteurization require temperatures of 75–90°C at the core of the product for several minutes, achieved by using an air temperature of 98–108°C, according to the shape. These conditions are nevertheless not enough for destroying the most heat-resistant microorganisms (i.e., spore-forming bacteria), unless the treatment is prolonged for a period of time that would be incompatible with the maintenance of the sensory characteristics typical of a "fresh" product. It is therefore important to apply preventive measures in order to keep the bacteria in the raw materials and the dough under control (Mondelli 2003a).

Figure 17.17 *Pasteurization tunnel. (Courtesy of STORCI, Italy).*

Pasteurization is followed by a surface-drying stage, an important step for reducing pasta humidity and a_w levels as required by law. The formation of a fine dehydrated layer on the surface of the product also avoids adhesion between the pieces.

The subsequent cooling phase is a very delicate one because the air must be filtered to prevent contamination of the product. Rapid cooling is highly preferable (Mondelli 2003b), as it limits the time that the product is exposed to temperatures favorable to microbial development. If the process does not provide for further heat treatments, the product temperature is then set to 4°C before packaging (this operation is discussed in Section 17.2.3.5).

The packaged product can be subjected to a second heat treatment, which can be omitted when there is no recontamination of the product after the first pasteurization and before packaging in a protected atmosphere. As well as giving priority to the hygiene characteristics of the raw materials and the working environment during the initial process operations, some industrial producers have elected to perform the final cooling and packaging in a clean room. The fresh pasta thus obtained has a flavor that is similar to products that have not been subjected to heat treatment, but it has a shelf-life of up to 60 days. Pasta obtained from modern technology can therefore guarantee a greater quality and safety than artisan products.

A second pasteurization treatment is often performed on sealed packages in an environment of 95–98°C for 40–50 min, in order to ensure a treatment at the core of the product of 80°C for about 10 min (Batisti and others 1995). The process can be discontinuous or continuous (with hot air or steam), and completed with a cooling phase. A more recent method with a lower impact on the pasta sensory and nutritional characteristics involves the use of microwaves in a tunnel where trays are transported on a conveyor belt at a set speed and exposed to temperatures over 75°C for several minutes.

The heat treatments, which are indispensable for ensuring health safety characteristics, give rise to important modifications to the protein and starch macromolecules, with interesting consequences on the rheological properties and behavior in cooking (Pagani and others 1999; Alamprese and others 2005a). As illustrated in Figure 17.18, after pasteurization, the pasta sheet appears less extensible and clearly stiffer due to the denaturation phenomena of gluten and egg proteins. This result reduces the significant heterogeneity of the texture (assessable via tensile test until the breakage of the product) that characterizes untreated fresh pasta. Cooking (Figs 17.18 and 17.19) restores flexibility to the texture of the product following a high absorption of water. In pasteurized products, where the protein network cannot be deformed as it is denatured and more efficient at

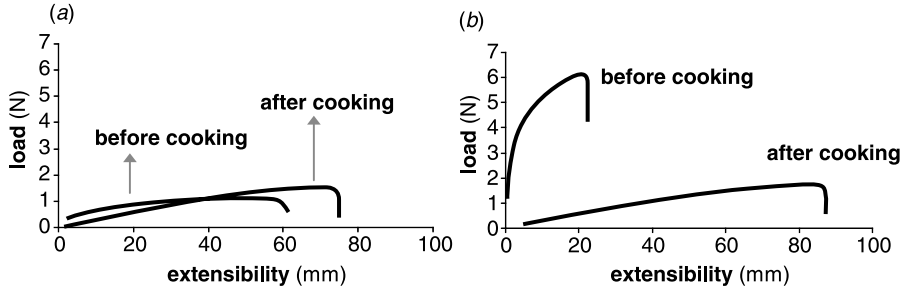


Figure 17.18 *Mechanical properties of pasta before pasteurization (a) and after pasteurization (b).*

preventing the swelling and solubilization of the starch material, the cooking loss is less than in the nontreated product (Fig. 17.20).

17.2.3.5 Packaging. In recent years, packaging has undergone highly interesting developments and innovations, which have favorably influenced product characteristics. Health characteristics have been improved too, lengthening shelf-life considerably, with a resulting increase in consumption.

The aerobic and microaerophilic microorganisms (*Lactobacillus*, *Staphylococcus*, *Bacillus*, yeasts, and moulds) find the best conditions for their development on the surface and inside the product, even at refrigeration temperatures. The removal of oxygen from the environment that surrounds fresh pasta can therefore control and significantly delay the development of these microorganisms without having to resort to drastic pasteurization or sterilization treatments that would damage the flavor and compromise the image of the fresh product.

Towards the end of the 1980s, several technological solutions were put forward based on vacuum packaging (Castelvetri 1991a), but were soon abandoned mainly because of the negative changes caused to the appearance of the product. The modified atmosphere packaging (MAP) technology involves the substitution of air in the fresh pasta packaging with a gas mixture in different proportions, mainly nitrogen and

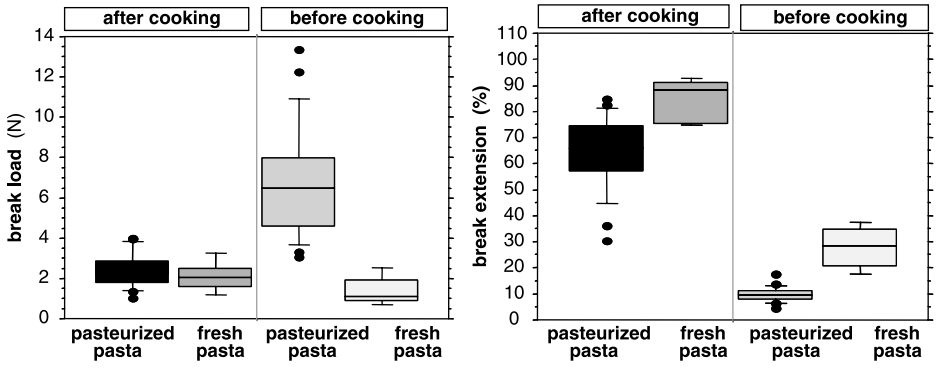


Figure 17.19 *Mechanical properties of pasta before and after cooking.*

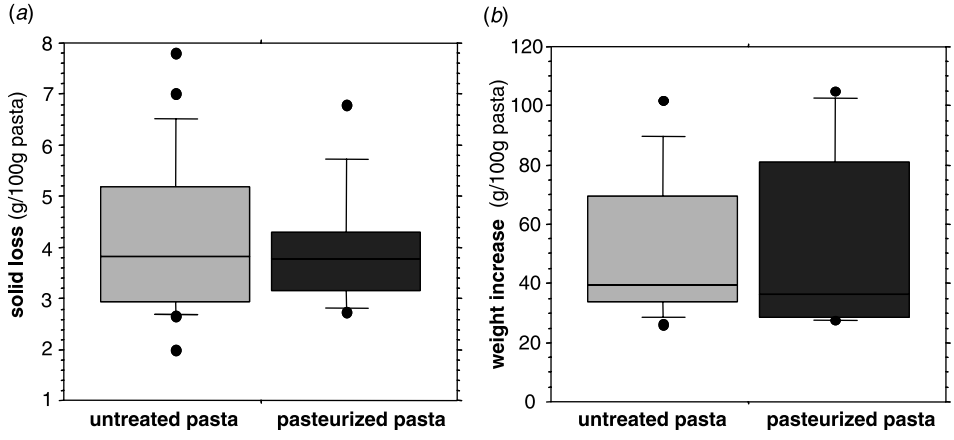


Figure 17.20 *Effect of pasteurization on cooking quality of pasta.*

carbon dioxide, and potentially, argon, helium, and nitrogen protoxide. These gases are called "packaging gases" by the Italian Legislation for Additives (Ministerial Decree 1996). This Decree allows this packaging technique to be used for all food products, without any restrictions.

The EC Directive 94/54 (1994) for food product labeling has introduced the term "protective atmosphere". The use of this practice (but not the gases used) must be written on the label. The use of MAP must not be seen as a remedy or means of improvement of a poor-quality food, but rather as a support technique that can have positive results only if combined with other technologies such as refrigeration (Squarzoni and others 2001).

The primary objective of MAP is to maintain a concentration of oxygen not higher than 1% inside the food packaging (Zardetto 2004). As well as preventing the risk of microbial development, the absence of oxygen in the packaging guarantees the maintenance of the sensory characteristics of the filling ingredients (eggs, meat, spinach, or cheese), which are highly sensitive to oxidation. Although this result can be achieved by substituting environmental air with nitrogen, it is useful to use a certain proportion of carbon dioxide that has a specific toxic effect on all aerobic microorganisms (Castelvetri 1991b; Farber and others 1993).

The percentage of CO_2 varies according to the process used (if pasteurization is made after packaging the CO_2 amount can be lower), and the characteristics of the product, that is, its tendency to release this gas. Generally, a proportion of CO_2 to N_2 equal to 30 : 70 is used, even though a better stabilizing effect is obtained with 50% CO_2 (Castelvetri 1991a).

However, these quantities are often associated with the formation of bubbles on the surface of the product at the time of cooking (Table 17.7). This defect can be traced back to the dilation of the gas, which has no way of being released during cooking (Sensidoni and others 1994). Although this phenomenon does not have any negative effects on the hygiene of the product, it is not appreciated by the consumer and so considered as a valid reason for rejection.

The selection of the packaging material is very important and must be made bearing in mind

1. The strict standards of barriers required;
2. The need to maintain the sensory characteristics typical to product; and
3. The heat-resistance level of the packaging in case it has to be subjected to a further heat-treatment (second pasteurization).

Plastic materials are used (envelopes or flexible or semirigid trays), with the exception of very short shelf-life fresh pasta, which is packaged using nonbarrier plastic materials. An efficacious barrier against O_2 and CO_2 is required when MAP is used. These properties are guaranteed by using co-extruded plastic materials, generally polypropylene (PP), polyamide (PA), polyethylene-terephtalate (PET), and ethylene-vinylalcohol (EVOH) (Stollman and others 1994).

Among the most interesting innovations in the field of fresh pasta packaging, have been made by Giavedoni and others (1994) on the possibility of further extending the shelf-life of fresh pasta with MAP packaging by the introduction of ethanol vapors.

17.2.4 Heat Damage to Pasta

Food quality is directly influenced by the intrinsic chemical and physical characteristics of the raw materials and also by the conditions of the processes used in each phase of the technological cycle. The heat treatments that food products are subjected to often give rise to undesirable changes in the color, flavor, and chemical macromolecules of the goods, and can also affect the availability of some nutrients. All these different changes have been covered by a single term, "heat damage". In a system that contains both proteins and reducing sugars, as in the case of cereal products, heat damage is mainly attributed to the Maillard reaction, or nonenzymatic browning (Nursten 2005; International Maillard Reaction Society 2006). The Maillard reaction is a complex reaction with two distinct stages, the early stage and the advanced stage (Friedman 1996). In the first stage of the reaction, part of the lysine molecule in the food product is chemically blocked and is not available from a nutritional point of view (Finot and Mauron 1972). At this point, this phenomenon is not yet accompanied by the formation of the molecules responsible for the flavor and color that distinguish the advanced phase and give the name to the reaction as a whole.

Evaluation of the heat damage is held to be highly significant nowadays as it makes it possible to complete the description of the foods' properties and distinguish and classify similar products in different quality classes. This criterion has already been applied with success to the dairy industry (Resmini and others 1993a).

In the previous sections, we have shown how the production of pasta, both fresh and dry, includes heat treatments at different temperatures and times. The extension of heat damage therefore contributes to a wide range of a variabilities) (Fig. 17.21). Quantification of this phenomenon is possible through establishing the dosage of particular molecules, the most widely known of which is furosine (Resmini and others 1990), a marker in the initial phase of the Maillard reaction. The intensity of the secondary phase in the pasta system is well illustrated in other neoformed products, both soluble (Resmini and others 1993b) and insoluble (Resmini and Pellegrino 1994).

As well as producing a negative effect on a number of the sensory characteristics of pasta, these changes also cause a decrease in the amount of bioavailable lysine (up to

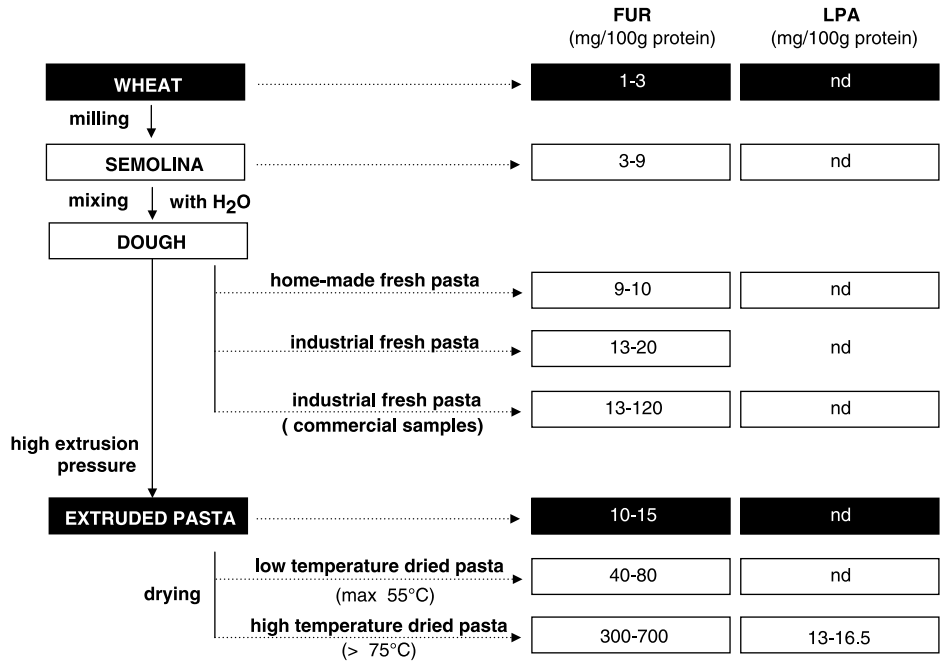


Figure 17.21 *Quantification of heat damage in different pasta products (FUR = furosine; LPA = lysylpyrrolaldehyde; nd = not detectable).*

40% of the original amount present in semolina; Resmini and others 1990), and the appearance of neoformed compounds that are typical of the crust of baked products (Resmini and Pellegrino 1994). Without doubt, the phase in pasta-making that is the greatest culprit when it comes to heat damage is the drying process. The intensity of the reaction is controlled by the drying diagram parameters (temperature, time, and pasta moisture) (Pagani and others 1992). In particular, the HT cycles widely used nowadays, thanks to the numerous benefits obtained with regard to behavior in cooking as mentioned above, can produce considerable heat damage, especially if the high temperatures are applied in conditions of low moisture (less than 15–17%) (HT-LM cycles). These conditions correspond to optimal a_w levels for the Maillard reaction.

These authors (Pagani and others 1996) demonstrated that each phase of the pasta-making process before the drying phase is associated with changes that directly or indirectly control the concentration of the molecules involved in the Maillard reaction. Special significance should be given to the glucidic fraction, which is the most highly affected with regard to quantitative variations. The critical parameters are those that influence the concentration of soluble sugars in extruded pasta, such as amylase activities and the mechanical damage to the starch granules during milling.

Even with fresh pasta, the Maillard reaction can vary over a wide range (Fig. 17.21); some industrial products are subjected to a double heat pasteurization treatment that is almost a "precooking" treatment (Mondelli 2003b), showing furosine levels that are 3–4 times higher than in fresh artisanal products.

It is difficult for the consumer to quantify heat damage directly, but this information could be included in the nutritional label given on the packaging. Although nutritional loss of lysine can be recovered through seasonings, sauces, and dressings, it is important

to state that the addition of animal proteins is considerably more costly than keeping the original proteins of the product. Future innovations in this sector will have to take these factors into account.

17.3 BREAD AND OTHER SAVORY PRODUCTS

It is difficult to establish who developed the first bread-making process. On the other hand, it is clear that both bread-making formulations and conditions have been handed down for millennia, continuously improving through to the present time. Bread production continues to be a complex and sophisticated process to this day, requiring much time, attention, and experience to guarantee good results.

The generic term "bread" embraces hundreds of different types of this product, each reflecting the traditions and cultures of the different countries of origin (Pomeranz 1987). Italian consumers prefer their bread with a crispy, highly fragrant crust and a soft crumb, with innumerable alveoli of different shapes and forms. The enormous difference in the humidity of the crust and that of the crumb (between 3–6% and 38–45%, respectively) and the preference for formulations with negligible percentage of sugars and fats (ingredients with the strong task of slowing down the staling process; Gray and Bemiller 2003) result in an extremely short shelf-life, a few days at most. This fact partly justifies the widespread presence of artisan bakeries throughout Italy (Pagani and others 2006).

For Anglo-Saxon populations on the other hand, the presence of a crust is considered a defect and the crumb must have fine, regular "bubbles" (Cauvain and others 1999). These characteristics can be clearly seen in pan bread, whose formulation includes antistaling ingredients and additives that slow down ageing and prolong shelf-life (longer than 2 weeks).

Some phases of the production process have a decisive influence on the product characteristics. In particular, the leavening of the dough due to yeast activity is fundamental not only to the formation of a soft, porous texture (which will be completed and stabilized during baking), but also to the production of metabolites that determine the taste and fragrance of the product. We can safely say, therefore, that bread production is one of the oldest, if not the first example, of food biotechnology.

Although critical to the bread structure, the leavening is not the only phase responsible for the creation of alveoli. The technological process of bread making, as for all baked-product processes, comprises a sequence of operations: mixing and kneading, leavening, and final baking in the oven. All these operations are indispensable for creating, maintaining, and "setting" the millions of gas bubbles in the dough and giving a product with a soft texture that is pleasant to chew (Dobraszczyk and others 2001). The wide variability of conditions in which each one of these phases can be carried out is responsible for the huge number of bread types available in Italy (Pagani and others 2006). In this chapter, we will highlight the main distinctive features common to Italian breads that can be found at the formulation and technological process level.

17.3.1 The Raw Materials

17.3.1.1 Wheat Flours and Flours from Other Cereals. The basic ingredients required for making dough for bread making are wheat flour, water, and yeast. Both soft wheat flour and durum wheat semolina are used in Italy. The widespread consumption of semolina bread in the regions of southern Italy is directly linked to the availability of

this product in that area (ISMEA 2002). Common wheat flour is, however, held to be the optimal raw material for bread making, because it gives the best leavening results (Hoseney 1989). This result does not depend on the quantity of the gluten proteins, which on average are higher in semolina than in remilled semolina (Table 17.2), but on the viscoelastic properties of the gluten network (Goesaert and others 2005). The flours with the best bread-making performance must contain insoluble proteins able to produce a dough with a strong yet extensible texture that is able to withstand the physical stresses of kneading and leavening. In other words, the most suitable flours for bread making must have an appropriate balance between dough viscosity and elasticity/strength (Belton 1999; Goesaert and others 2005).

The many works on this subject all agree that the key factors are the gliadin/glutenin ratio and the structure, composition, and molecular weight of the different subunits in the protein fractions (MacRitchie 1992). In particular, the high-molecular-weight (HMW) glutenin subunits have a key role in defining the strength characteristics of the dough, as discussed in a recent work by Shewry (2003).

The study of the properties of the macromolecules and the biochemical mechanisms involved in the formation of the dough is therefore indispensable in the search for new varieties of wheat of optimal bread-making quality. Nonetheless, the identification of these new types of wheat by operators in the sector is usually based simply on observation of the behavior of the flour during rheological tests defined as descriptive or imitative because they simulate the strain exerted during the bread-making process (Dobraszczyk 2003). The information obtained from the so-called fundamental rheological tests are more difficult to correlate to the behavior of the material during a real production process (Dobraszczyk 2003).

The most frequently used voluntary policy in Italy (Aldovrandi and Vitali 1995; Table 17.2) for defining the classification of commercial wheat flours provides for five classes, as a function of the total protein content and the main rheological indices, such as the alveographic indices (W and P/L) and farinographic stability. Information obtained from the rheofermentograph (Mariotti and others 2006b) is also being increasingly used. This test completes the description of the behavior of a flour in bread making by supplying data regarding the development of the mass due to CO_2 and the quantity of gas actually retained in the dough.

The durum wheat doughs are distinguished by a high resistance to deformation and consequently limited extensibility, characteristics typical to the proteins of this raw material (Boyaciogly and D'Appolonia 1994; Pogna and others 1996), which allow only a modest development in mass volume during leavening. To be judged suitable for bread making, the semolina must have a high protein content ($>12\%$), a good farinographic stability, and alveographic P/L index values below 1 (Boggini and others 1995, 1997).

It should also be noted that the bread-making process preferred in the case of durum wheat requires the use of sourdough. A case in point is the *pane di Altamura*, Altamura Bread, whose process is described in detail in Section 17.3.3. The proteolitic activities of semolina and/or of the lactobacilli may produce considerable changes in the rheological properties of the gluten network, reducing the natural excessive elasticity of dough made from durum wheat (Martinez-Anaya 1996; Brandt and others 2003; Clarke and others 2004).

The availability of cereal flours other than wheat flour in the mountainous and hilly areas of Italy, in particular rye, barley, oats, and corn, justifies the extraordinary variety of bread formulations existing in this country. A survey of the many existing bread formulations was recently made by Bordo and Surrasca (2002) and INSOR (2000). Today, the

use of flours other than common wheat for bread making is being seriously reconsidered, because their use would improve the nutritional properties of the food (Pagani and others 2002; Shfali and Sudesh 2002). However, this practice produces dough systems with special rheological properties and technological performance (Dendy 2001; Mariotti and others 2006b). Raw materials such as rye or barley, whose proteins have a lower capacity for assembling into a viscoelastic network than wheat gluten, provide mixtures with a low expansion capacity for developing during leavening (Panigone 2004). The low bread-making quality of these cereals can be improved with the addition of wheat flour having superior gluten performance. This solution makes it possible to obtain a sufficiently developed product (Fig. 17.22). The presence of high percentages of fiber in these cereals also ensures a prolonged shelf-life (Katina 2003).

The bread-making performance of wheat mixtures enriched with gluten-free flours such as corn or oats flours is also critical. The "dilution" of gluten has a negative influence on the formation of a regular network and consequently on the development of the volume of the product (Zhang and others 1998; Dendy 2001). A number of studies by Mariotti (2004) and Pagani and others (2002) have shown that mixtures of top quality bread-making wheat flour enriched with 40% buckwheat flour or toasted oats give satisfactory results with regard to volume development proportional to the bubble's area during leavening and baking (Fig. 17.23). It is possible to optimize the formulation by adding 5% puffed buckwheat flour (PBF) with consequent improvement in the shelf-life of the bread, which maintains its fresh-bread softness for longer (Mariotti 2004) (Fig. 17.24).

17.3.1.2 Yeast. The term baker's yeast is used to refer to *Saccharomyces cerevisiae* strains selected for their fermenting powers in bread making. At the end of the kneading phase and after an initial rest phase (a brief period of only several minutes, during which the oxygen in the microbubbles dispersed in the dough is consumed), the microorganisms start fermenting, first using the free sugars in the flour (glucose, fructose, maltose, and so

FORMULATION	Bread characteristics(*) height (mm)	diameter (mm)	volume (mL)
100% wheat flour (WF)	52	99	280
30% barley flour + 70% WF	52	81	196
30% corn flour + 70% WF	42	100	199
30% coarse corn flour + 70% WF	39	106	188
30% whole rye flour + 70% WF	47	93	214
50% rye flour + 50% WF	61	82	241

(*) *starting from 100 g of dough*

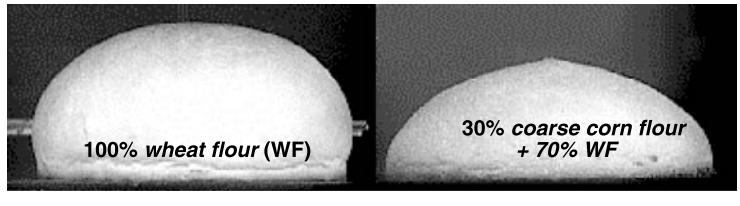


Figure 17.22 *Effects of various formulations on bread characteristics.*

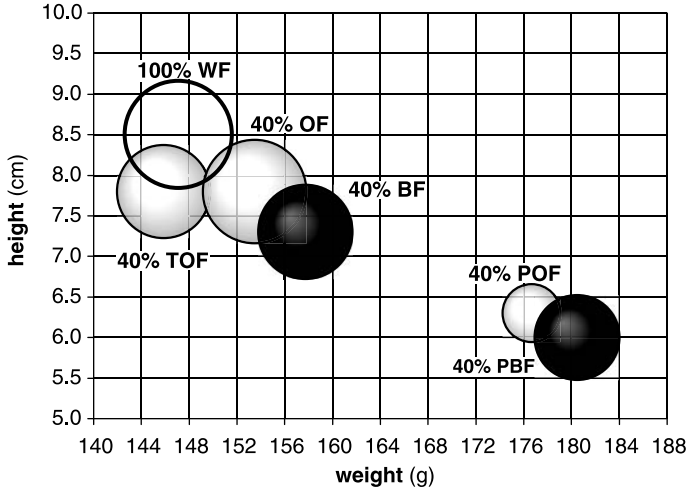


Figure 17.23 *Characteristics of bread from different mixtures of flour (WF = wheat flour; OF = oat flour; TOF = toasted oat flour; POF = puffed oat flour; BF = buckwheat flour; PBF = puffed buckwheat flour).*

on) and then those freed by the endogen amylase activities or by the consequent addition of enzymes.

From the industrial production process of bread-making yeast, we obtain a cell suspension in water of 18–20% yeast solids, called yeasts cream. This biomass is cooled to 2°C to block all metabolic activity and filtered using a rotating drum vacuum filter to produce compressed yeast (approximately 26–28% yeast solids), which is usually shaped into cakes. The product is left to stand in a cold-storage room for several days awaiting laboratory analyses, including the evaluation of the reserve carbohydrates trealose and glycogen, which are good quality markers. The color of the compressed yeast depends on the substrate used for its production and the pretreatments applied to molasses to reduce

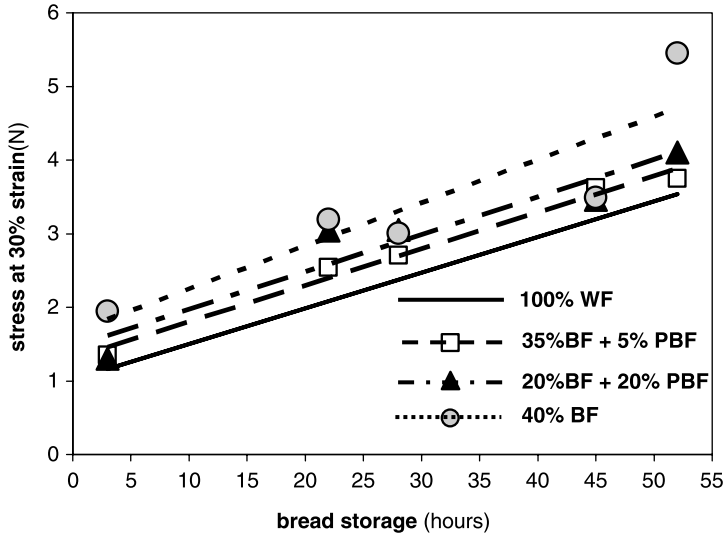


Figure 17.24 *Measures of shelf life of bread for different flour mixtures (WF = wheat flour; BF = buckwheat flour; PBF = puffed buckwheat flour).*

the tannin and fiber content. Compressed yeast can be subjected to an extrusion process and subsequent drying. In this way, we obtain a dried form that is easier to preserve and has a longer shelf-life. However, the product requires a rehydration phase (e.g., at 30–40°C for 10–15 min) before being used for bread making.

Dried "instant" yeasts with morphological and structural characteristics that preclude the rehydration phase and so can be mixed directly with the dry ingredients have been on the market since the 1960s. These are small, yet highly porous granules, with a high specific surface (Pointrenaud 1994). In addition, the genetic selection of the microorganisms has made it possible to obtain strains that can be dried, but maintain their good fermenting capacities. Recently, yeast in the yeast cream form has also been used.

The different types of yeasts differ in temperature and storage time, which varies between 3 and 4 weeks for the compressed yeast and up to 12 months for the dried yeast. According to the law (Italian legislation, Presidential Decree 1998), yeast sold for bread making must contain a maximum part of vital cells with an adequate fermenting power, and humidity and ash not exceeding 75% and 8%, respectively (on a dried basis), for the compressed yeast, and 80% and 8% for yeast cream.

17.3.1.3 The Other Ingredients. The large number of types of bread produced in Italy vary greatly, depending on the kind of flour and the quantity of water used. The amount of water used varies widely, between 40 and 70 parts per 100 parts of flour (Pagani and others 2006). This parameter has a significant effect on the rheological properties of the dough and consequently on the bread crumb grain. A low-humidity dough (about 35–40% water) is firm and stiff and the gluten presents a low extensibility: during the leavening phases, the expansion is considerably limited, giving the bread a compact crumb texture, like in *pane di pasta dura* and *pane Ferrarese*. On the other hand, a high-humidity dough (65–70% water), characterized by a relevant extensibility, cannot be molded into regular shapes and the CO_2 developed during leavening tends to collect into large bubbles typical of the irregular, flat loaves called *Ciabatta*, which have an uneven grain (Pagani and others 2006).

The action of water is indispensable for the formation of gluten. It also acts as a solvent for the other ingredients such as salt and sugars, and promotes the enzymatic activities of the flour, both those that are endogenous and those that are added enzyme to improve the behavior of the dough, especially during leavening. Water also contributes to the swelling of the starch grains during baking, and their gelatinization, a key phase for the development of both the physical properties of the bread and its nutritional value (Eliasson 2003).

In the artisanal preparation of bread, other additives are added such as malt extract, malted cereal flour, and ascorbic acid (Italian Legislation 1998). The use of malt and/or malted cereal flours enriches the dough with enzymes, especially amylase, which hydrolyzes the starch into fermentiscible sugars, a substrate for yeasts during the fermentation phase. There is a rapid start to the leavening, with an increase in the product final volume and consequent improvement in the alveoli structure (Goesaert and others 2005). Ascorbic acid is added (the Italian Legislation quoted does not specify the "maximum quantity" but says "enough": it is a question of parts per million), thanks to its ability to promote the formation of sulfur bridges between the gluten proteins that increase the strength of the dough (Goesaert and others 2005).

Other optional ingredients are shortenings, such as butter, lard, or olive oil. In order to be able to include these ingredients in the name of the bread (e.g., *pane all'olio*, oil bread),

they must constitute at least 3% of the dry material in the finished product. The main technological role of shortening, when present in small percentages, is as a lubricant. Shortening facilitates the flow among the gluten macromolecules, which will have a greater extensibility (greater volume of the final product) (Desgrets 1994). In addition, a number of the lipid fractions stabilize the air bubbles created during kneading, ensuring a more regular alveoli of the crumb. Lastly, during storage, the lipids prevent interaction between the starch granules, slowing down starch retrogradation and blocking water migration between starch and proteins, consequently slowing down the staling process (Gray and Bemiller 2003).

17.3.2 Bread-Making Process

17.3.2.1 Bread-Making Processes with Baker's Yeast. Bread production is mostly made up of unit operations as shown in Table 17.8, which also summarizes the specific objectives and modifications of each technological step. A fundamental difference, which distinguishes almost all breads produced in Italy from breads produced in Anglo-Saxon countries, is the absence of a pan used in the leavening and baking phases. Not using a pan gives the possibility of shaping the bread into endless numbers

TABLE 17.8 Main Phases of the Bread-Making Process and Related Modifications.

Phase	Aim	Modifications
Mixing	• Distribute ingredients homogeneously	• Hydration and solubilization of the water soluble compounds
	• Form a uniform and "coherent" structure	• Formation of gluten
	• Include air bubbles	• Inclusion of microbubbles
Leavening	• Increase volume of dough	• Formation of gas (CO_2)
	• Develop typical "flavor" characteristics	• Production of fermentation metabolites important for developing flavor and changing macromolecule solubility
Shaping	• Subdivide gas bubbles and include new air	• Division of dough into final pieces
	• Give shape to dough	
Baking	• Decrease water content	• Increase in volume due to evaporation of gases: 20–30% of the volume is obtained during baking (oven-spring)
	• Fix the leavened and shaped dough	• Formation of crust and crumb
	• Make product appetizing and digestible	• Denaturization of proteins
	• Give the product its typical coloring	• Gelatinization of the starch
		• Development of flavor
		• Evaporation of water and ethanol
Cooling	• Product packaging	• Change of sugar solubility
		• Hardening of fats

of shapes and sizes, with the most sophisticated and refined reserved for special occasions (Bordo and Surrasca 2002).

The production of bread in Italy is traditionally a discontinuous process, because each phase of kneading, leavening, and baking is conducted on a moderate quantity of material and there is a separate machine for each. The process is typically artisanal in every region. The number of bread-making laboratories, often family-run, totals about 30,000 (Pagani and others 2006).

The discontinuous bread-making processes are performed using the straight-dough (in Italian *metodo diretto*) or the sponge and dough (*metodo indiretto*) processes; sourdough-bread-making can be considered as a particular sponge and dough method (Fig. 17.25).

With the straight-dough method, all the ingredients are mixed together at the same time to form a dough, which is then left to rise. The yeast is composed of selected cultures of *Saccharomyces cerevisiae*. Fermentation must occur in at least two phases. The first fermentation, called *puntata*, is generally effected on large masses of dough, at variable periods of time from about 30 minutes to three hours, according to the process. The objective of this operation is not to obtain an increase in volume, but to induce important changes in the rheological properties of the dough (Chargelegue and others 1994). In particular, the solubilization of part of the CO_2 produced lightly acidifies the system and causes the gluten to become stronger. This phenomenon improves the kneading of the dough and its ability to maintain the shape given during the second fermentation or *appretto*. In this phase, the dough is given its final shape and left for an hour under controlled temperature and humidity until it has reached its maximum volume. The advantage

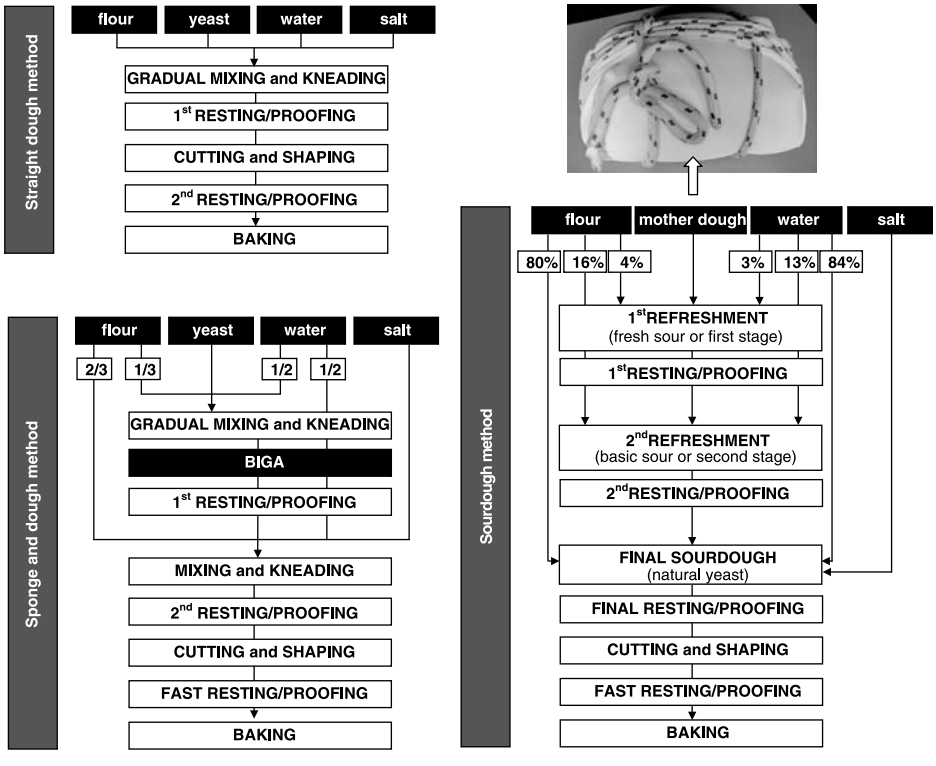


Figure 17.25 *Different methods of bread-making.*

of using the straight-dough method, thanks to its speed and use of compressed yeast, is the possibility of using lower-strength flours, because a shorter leavening time is required.

In the sponge and dough processes, the ingredients are added at different times (Fig. 17.25). These processes are still widely used in Italy, both at the artisanal and industrial level (Pagani and others 2006). The processes include the formation of a preparatory sponge, called *biga*, which involves mixing part of the compressed yeast (selected *Saccharomyces cerevisiae* strains), part of the flour, and part of the water required for the entire formulation. After a holding period (which can vary from 3–4 to 20 hours according to the type of bread desired), the remaining flour and water are added, and then the remaining ingredients. During the long rest phase, the yeast adapts itself to the dough-system, and reaches optimal fermentation capacity when all the ingredients have been added. After 30–60 min of leavening, the dough is cut, shaped, and left to rise again for about an hour, and is then baked.

The long leavening times required for the sponge process provide, in the final product, a crumb grain texture with a high number of bubbles (Brown 1993), often with an irregular size distribution. This alveolar structure provides greater initial softness, as indicated by the lower consistency, and longer storage times with respect to the product obtained with the same raw material using the straight-dough process. After 24 hours, the crumb hardness of a straight-dough loaf is 50% higher than that of a sponge-and-dough loaf (Pagani and others 2006).

17.3.2.2 Bread-Making Processes with Sourdough. Sourdough bread-making processes are still widely used in Italy. Without doubt, they can be considered one of the most delicate and complex transformations found in food technology.

As recently summarized by De Vuyst and Neysens (2005), referring to the classification of Böcker and others (1995), sourdough is conventionally subdivided into three types on the basis of the preparation method.

Type I Sourdough or Traditional Sourdough. At present, this is the most widely used type of sourdough used in the production of traditional breads. The process is characterized by continuous steps at environmental temperature (22–28°C) and ~pH 4, with the objective of keeping the microbial population vital and constant, both from the point of view of the number of microorganisms and also the number of species. Sourdough contains both yeasts and lactobacilli. In particular, type Ia sourdough is composed of a well-adapted microflora and maintains a constant composition. Type Ib sourdough requires regular fermentation or refreshment phases (fresh sour, basic sour, and full sour, Fig. 17.25) in order to maintain the dough microbial association unchanged over time (even decades). The mother dough or starter represents the starting point (inoculum) of every bread-making process. Type Ic sourdough refers to products at higher temperatures; a case in point is the sorghum dough, typical of a number of African countries.

Type II Sourdough or Accelerated Sourdough. This is a semifluid dough developed to satisfy the requirements of industrial processes that demand fast and controllable techniques on a large scale. They are obtained at temperatures over 30°C and generally have a shelf-life of several days (approximately one week in a cold environment). During the preparation of these sourdoughs, the microorganisms are in a stationary phase and have limited metabolic activity. This procedure requires the addition of

Saccharomyces cervisiae, of commercial origin, which is responsible for the increase in volume during leavening, an operation performed in a single stage.

Type III Sourdough or Dried Sourdough. This dough is obtained by using commercially dehydrated starters aimed at giving acidity and fragrance to the product. The type III dough also requires the addition of *Saccharomyces cerevisae* to guarantee leavening.

As already mentioned, regardless of the process adopted (artisanal or industrial) and of the formulation (savory as in the case of bread or sweet as in the case of festivity cakes), most Italian sourdough products are obtained using type Ib sourdough (De Vuyst and Neysens 2005; Pagani and others 2006). These products require inoculum (mother), that is, the addition of a piece of dough from the previous day. This primary dough, which represents 5–20% (w/w) of the final dough, goes through several refreshment or backslopping phases under controlled conditions to obtain the full sourdough after a single or multistage process (Spicher 1983; Ottogalli and others 1996; Vogel and others 1996). Each refreshment stage causes a significant increase in the leavening mass and its fermentation capacities (Pagani and others 2006).

The type Ib sourdough contains several lactic acid bacteria (LAB): works on this subject refer to over 50 LAB species of *Lactobacillus*, *Pediococcus*, and *Leuconostoc* in concentrations of about 10^8–10^9 cfu/g (Gobbetti and others 1994; Ottogalli and others 1996; Stolz 2003; De Vuyst and Neysens 2005). The microbial groups are linked in a noncompetitive and often mutualistic equilibrium, forming an ecosystem that is quite stable to external perturbation (Martinez-Anaya and others 1990; Foschino and others 1995; Gobbetti 1998; Stolz 2003; De Vuyst and Neysens 2005). Generally, sourdough contains yeasts of the *Saccharomyces* and *Candida* species, which are responsible for alcoholic fermentation and consequently for the development of the volume of the dough. The yeasts are in association with the LAB in a ratio of 1:100 (Ottogalli and others 1996; Stolz 2003). The LAB perform an intense acidifying activity, producing lactic acid and acetic acid in quantities related to the species present in the sourdough (obligate homofermentative, facultative heterofermentative, and obligate heterofermentative). They are capable in any case to determine both the significant increase of the total acidity and the decrease of pH (Foschino and Galli 1997; Stolz 2003; Gobbetti and others 2005). Characterization of the acid dough can be completed by calculating the so-called fermentation quotient (FQ), that is, lactate/acetate molar ratio.

These changes not only influence the sensorial properties of the dough and the finished product (Spicher 1983; Schieberle 1996), but also their consistency (Crowley and others 2002), thus providing an extended shelf-life (physical and microbiological) of the bread obtained using this process (Röcken 1996; Corsetti and others 1998; Raffo and others 2002, 2003). Equally important for assuring fragrance and prolonging shelf-life are the metabolites produced by the amylase activities (sugars and low-molecular-weight maltooligosaccharides with a degree of polymerization from 3 to 9) and the protease activities (peptides, amino acids) performed by the LAB, as illustrated in the review by Martinez-Anaya (1996) and in the work of Gobbetti and others (1996). The production of exopolysaccharides from cereal-associated LAB is also being studied with increasing attention thanks to the positive effects on the dough rheological properties and bread quality (Tieking and Gänzle 2005). Moreover, the changes in the hydration state of the gluten proteins promoted by the souring could also be involved in maintaining crumb freshness for a longer time (Crowley and others 2002). Nevertheless, the delay in bread

staling promoted by biological acidification may differ according to the lactic acid bacteria strains and the level of acidity obtained (Corsetti and others 1998, 2000). The extended shelf-life of sourdough bread is not only due to the slowing down of the development of mold and bacteria in an acid environment but also to the production of bacteriocins by a large number of LAB (Gobbetti and others 2005). The greater softness of the crumb with respect to bread obtained using the straight-dough method is partly due to the greater and more uneven alveoli (Brown 1993). This is associated with the slower and more gradual production of CO_2 and the merger of small bubbles into larger alveoli.

Bread obtained from sourdough has a characteristic flavor and fragrance due to the formation of volatile organic substances (fermentation byproducts) and fragrant substances formed during baking following the Maillard reaction between the glucose and amino acids produced during the long fermentation process (Schieberle 1996). It must be remembered that sourdough bread is more easily digestible due to the macromolecule-extended enzymatic hydrolysis action by the LAB that facilitates hydrolysis (Katina and others 2005).

As already mentioned, the development and maintenance of the microbial species typical to sourdough are achieved using traditional discontinuous processes that require subsequent refreshments of the primary dough in order to obtain the full sourdough. Although all producers apply a constant microorganism propagation process in their bread making, the sourdoughs differ both from the point of view of formulation and of storage conditions (Hammes and others 1996). These factors are conducive to specific symbioses between microorganisms with interesting repercussions on the sensorial properties of the finished product. All researchers engaged on this subject agree that *Lb. sanfranciscensis* can be considered the key bacterium in the sourdough process (Gobbetti and others 1996; Gobbetti and Corsetti 1997). In fact, together with *Lb. pontis*, it constitutes the major part of the microbial flora and establishes interesting trophic relationships with the sourdough yeasts (*S. cerevisiae*, *S. exiguus*, or *Candida milleri*) due to its effective maltose metabolism (Stolz and others 1993). The overall metabolic activities of these dominating bacteria assure a rapid acidification, a balanced production of lactic and acetic acid (as expressed by the FQ), and gas production.

17.3.2.3 Optimization and Innovation of the Sourdough Process. As we have seen, the sourdough processes require a long working time and considerable experience. In spite of the significant complexity of the process, this ancient technology is still in practice today, even at the industrial level. This is because it is able to produce bread and other sweet and savory products that combine high sensorial characteristics such as softness and lightness of crumb, and the typical fragrance and taste, with longer and easier storage.

For some time, proposals have been put forward for improving the process with methods that maintain the numerous advantages associated with acid dough and simplify the management of the technological cycle. The type II and type III sourdoughs may be considered effective examples in this sense.

As already seen, the type II sourdough is a semifluid silo preparation for use in continuous processes and guarantees a correct acidification level of the dough (Böcker and others 1995; De Vuyst and Neysens 2005; Decock and Cappelle 2005). The type II sourdoughs are obtained with a long process (2–5 days) at a temperature $>30^{\circ}$C aimed at bringing the microorganisms to the stationary phase, characterized by a limited metabolic activity. Under these conditions, *Lb. sanfranciscensis* is not sufficiently competitive to dominate the fermentation. The ecosystem is characterized by LAB of other genera and species, both homofermentative and heterofermentative. Yeasts are not found in significant

amounts; therefore, the addition of baker's yeast is necessary (Martinez-Anaya 2003) and the fermentation occurs using a long-term single step. As stated by Stolz and Böcker (1996), this approach has transformed traditional sourdough technology "from work-consuming multiple-stage processes (the type I sourdough) to work-saving one-stage processes", capable of guaranteeing greater flexibility and stability in the final characteristics of the product. Another important advantage of the type II sourdough is its low consistency, which makes the inoculum pumpable, resulting in a higher dough yield.

The evolution of this technology is represented by equipment that provides for continuous sourdough fermentation (Stolz and Böcker 1996; DeVuyst and Neysens 2005). This process has been adopted in Italy by a small number of producers of festivity cakes and breads at the industrial level. In this case, and similar to what has occurred in the dairy and meat sectors, the starter, often called preferment, is represented by LAB selected cultures (rarely in combination with yeasts), which are prepared by specialized companies and sold in the form of lyophilized granules. The production of these starters is shown in Figure 17.26. Starting with selected strains, an intermediate culture is obtained. The following fermentations are carried on in reactors. The biomass is then separated, washed with isotonic solutions, has added cryoprotectants, and is lyophilized. The industrial process continues with grinding of the lyophilized biomass, standardization of the viable cell count, and packaging of the finished product.

The concentration of vital microorganisms is extremely high (the LAB are greater than 10^9 cfu/g) (ANIDRAL Srl, personal communication). After a thorough and extended revitalization phase of the microorganisms in fermenters at 27–32°C, generally in the presence of flour, the liquid preferment is mixed with the remaining part of the ingredients, applying the all-in-one process (Stolz and Böcker 1996). The comparison between the traditional process and the preferment process not only highlights a saving of time, but also emphasizes the greater facility and standardization of the operations (Fig. 17.27).

Although the starters have significantly simplified the production of sourdough, this approach is still considered too expensive (because of the need for fermenters and the time required, from 16 to 24 hours to obtain the full sourdough) for small artisanal producers. These disadvantages may be overcome in part by type III sourdoughs or dried sourdoughs (*madri essiccate* in Italian). Products of this type are widely used in rye bread making because the high acidity level that distinguishes them inhibits the amylase activity typical of this cereal. Type III sourdoughs are commercial products that are obtained from mature sourdough and stabilized via spray-drying or drum-drying (Stolz and Böcker 1996). Another technological solution based on methods used in the past to preserve acid yeast is known as the "granulation process". First, the moisture content of the acid mass is lowered with the addition of an appropriate amount of flour, then brought to values lower than 15% via drying (BakeMark Italia, personal communication). Following the dehydration/drying phase, the microorganisms are no longer vital, but the products contain the metabolites of the microbial populations from which they were obtained. With the use of these starters, the fragrance and acidity properties that with a conventional process would be seen only after many hours of leavening can already be observed at the end of the kneading phase.

Similarly to what we have seen for the type II sourdough, the addition of baker's yeast is nevertheless indispensable for ensuring leavening capacity. Other advantages of dried sourdoughs include ease of use and high standardization, a longer shelf-life, and easy storage of the finished products. The most popular microorganisms for this process are

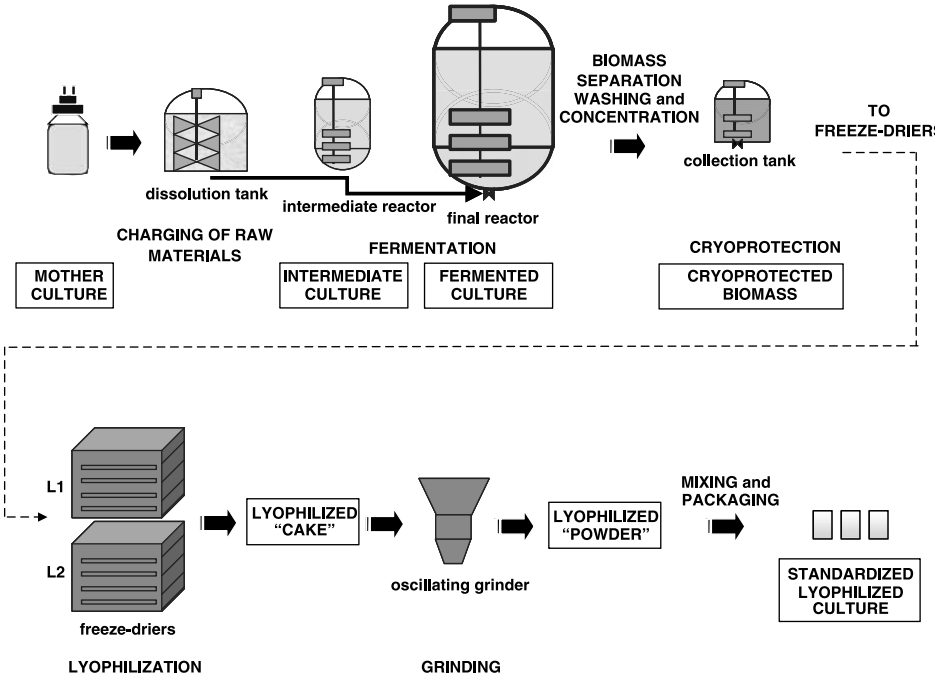


Figure 17.26 *Production of starters: fermentation reactors (a), lyophilizers (b). (Courtesy of ANIDRAL, Italy).*

Lactobacillus plantarum, *brevis*, *casei*, *diacetylactis*, *fermentum* and *Pediococcus acidilactici*, and *pentosaceus* species, because they have a greater resistance to drying (Martinez-Anaya 2003).

These commercial preparations can be classified on the basis of color, fragrance, and acid content. The acid content is extremely important, because it determines both the technological characteristics of the product and the fragrance. Dried sourdoughs can be found on the market with an acid value (expressed as mL NaOH 0.1 N used to neutralize 10 g of suspended sourdough in 100 mL water) that can vary between 15 and 60 and more according to the production process of the sourdough and the initial raw material (wheat flour or

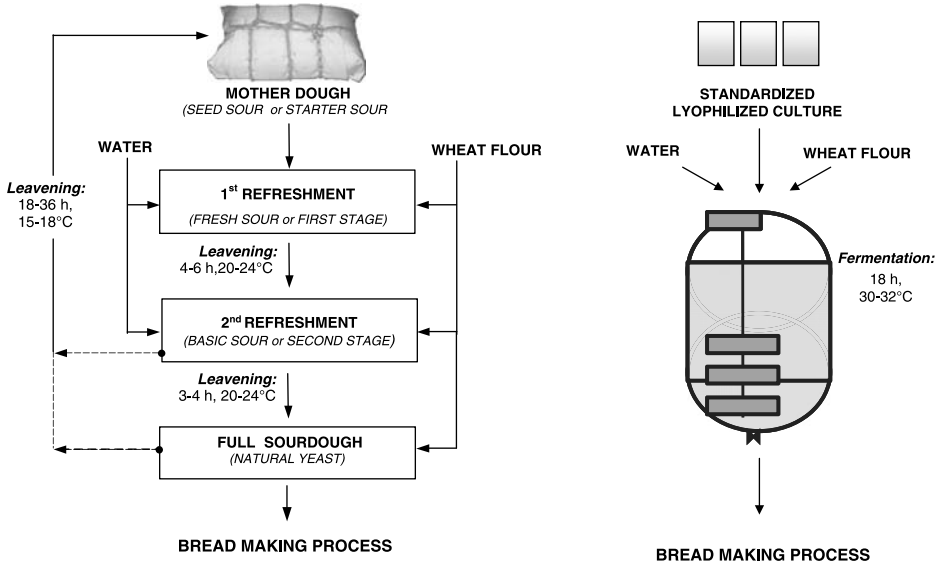


Figure 17.27 *Comparison between the traditional sourdough process and the preferment process.*

rye). As acidity is increased, a strong sour fragrance is developed and the color of the bread becomes more intense due to the catalyzation of the Maillard reaction in an acid environment. The use of dried sourdough with an acid value of 15–30 gives a more elastic and stable bread that is less sour, has a more delicate fragrance, and a more friable crust (Bake-Mark Italia, personal communication).

17.3.3 Process Card of Typical Italian Savory Products

Over the centuries, the experience and skills of Italian breadmakers have given rise to a very large number of types of bread. The Bordo and Surrasca (2002) and INSOR (2000) guides mention at least 200 types of bread still produced in Italy today. Each differs from the others from the point of view of formulation (presence of cereal flours other than bread wheat flour), size or shape, and process conditions, due in particular to the leavening and baking phases. Rightly therefore, the artisanal activity has been given the name "white art", as it has been successful in developing original methods for employing the raw materials found in such a heterogeneous territory as Italy to the greatest possible advantage.

A number of these methods have been used in industry, especially in the sweet baked products sector (Pagani and others 2006). In fact, the most well-known traditional Christmas products, *Panettone* and *Pandoro*, are today available on an industrial scale. Thus, it is possible satisfy the demands of a much larger number of consumers far from the region of origin of the product, guaranteeing low costs combined with high quality. The regulations applied by the Italian Legislation (2005) have set the minimum parameters for content as well as a number of process variables.

As an example of the complexity of the Italian baked products sector, we present here the "product and process cards" for a number of savory foods of this category. The first card refers to the *pane di Altamura*, Altamura bread, a typical durum wheat semolina

bread of ancient origin. There are extremely strict conditions to be complied with in order for *pane di Altamura* to be called such, and, consequently, it cannot be produced on an industrial scale.

The other cards refer to two products that belong to the "bread substitute" category, *grissini* (breadsticks) and *taralli*. Although their geographical origins are distant from one another (*grissini* are from northern Italy, *taralli* from southern Italy), they are similar with regard to consumption. Together with artisanal products, industrial products are also sold using the same names but with completely different distribution channels and shelf-life so as to satisfy the requirements of different kinds of consumers.

17.3.3.1 An Example of the Sourdough Process: Pane di Altamura. Historical sources date the beginnings of bread-making activity in Altamura to 1420, but Horace (37 A.D.) referred to bread in the murgiane area as the "best in the world".

The bread of Altamura (a small town not far from Bari) is a typical bread from the Puglia region and was the first bread in Europe to be recognized with the label of Protected Designation of Origin (PDO) (EEC 2003). The PDO denomination (DOP in Italian, *Denominazione di Origine Protetta*) is assigned to products that have a strong link with their region of origin. Two conditions must be satisfied to receive the PDO designation: production of the raw materials and their processing through to the finished product must be made within the region and meeting precise conditions. Furthermore, evidence must be provided that the special quality characteristics of the product are exclusively or essentially dependent on the geographical environment (climate, quality of the soil, human factors, and so on) of the place of origin.

The bread is obtained from re-milled semolina of four durum wheat varieties, Appulo, Arcangelo, Duilio, and Simeto, grown in the area of Altamura (Pasqualone and others 2002). These raw materials can be used on their own or in combination, making up at least 80% of the total semolina. The production specifications (see also www.panedialtamura.info) specify all the bread-making steps, which are described in every detail (Fig. 17.28 shows the flow-sheet and some operations of the process). The official recipe consists of 20 parts full sourdough to 100 parts durum wheat re-milled semolina, 2 parts salt, and 60 parts water. The full sourdough is prepared with three refreshment steps (type Ib sourdough). The final kneading takes 20 min and the mass is then covered with a thick cloth to maintain a constant temperature and left to rest for 90 min, generally on wooden slabs. The final shape is obtained via three distinct molding phases, each with rest phases (intermediate proof) of established times. This operation is still performed manually. The baking operation must also be performed under controlled conditions in ovens heated with oak wood in order to obtain bread loaves with the characteristic crust at least 3 mm thick, and the typical flavor, due both to the presence of durum wheat semolina and to the sourdough process.

Each loaf is placed into the oven, one at a time, with a 4 m-long baker shovel starting from the innermost part of the oven. Great skill and experience is necessary for placing the loaves into the oven quickly and systematically in order to use up the space in the most effective way, and ensuring that the loaves do not touch each other. A bread brand was used to mark the bread of each family. In the past, the baking of *pane di Altamura* was made exclusively in public ovens due to the fact that bread-making in the home was prohibited; violation of the regulation meant a huge fine. The families therefore, made the dough in the home and took it to the baker for baking. In order to distinguish between the loaves the families used wooden or metal brands bearing the family's initials or other symbols.

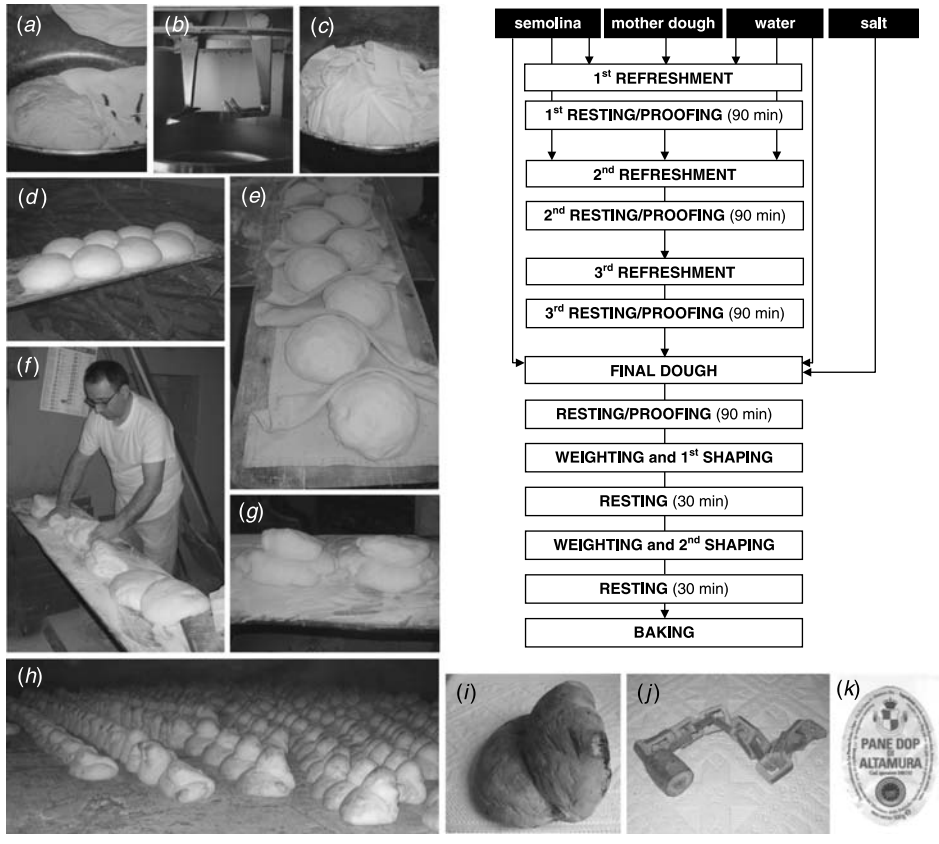


Figure 17.28 *Flow sheet and operations of the pane di Altamura bread-making process: mother dough refreshments (a, b, c); first shaping and resting (d, e); manual shaping of the dough (f); an example of Pane di Altamura dough shape (g); the baking phase (h); bread after baking (i); the ancient instrument used to mark the bread (j); the current "logo" of the Pane di Altamura DOP (k). (Courtesy of Panificio Di Gesù, Italy).*

The traditional shape of *pane di Altamura* resembles a hat with a wide brim (Fig. 17.28*i*). It is also produced in the form of a large loaf, which can weigh up to 2 kg. Its main characteristic, as well as the typical yellow color of the crumb due to the semolina pigments, is its long shelf-life of at least one week (Raffo and others 2002, 2003), equal to the time the farmers and shepherds spent with their herds before returning home.

17.3.3.2 Examples of the Straight and Dough Process: Grissini and Taralli. Both *grissini* (breadsticks) and *taralli* are dry oven-baked products characterized by a high friability and long shelf-life if suitably packaged to prevent absorption of water from the environment. The formulations of both products include added ingredients (fats, generally olive oil) which improve palatability and friability.

The differences lie in the raw material, common wheat flour used in *grissini* and re-milled semolina in *taralli* according to the region of origin and consequently the availability of one type of flour or another. *Grissini* or breadsticks first appeared in a region of northern Italy (Piedmont). This particular bread probably dates back to the middle of the

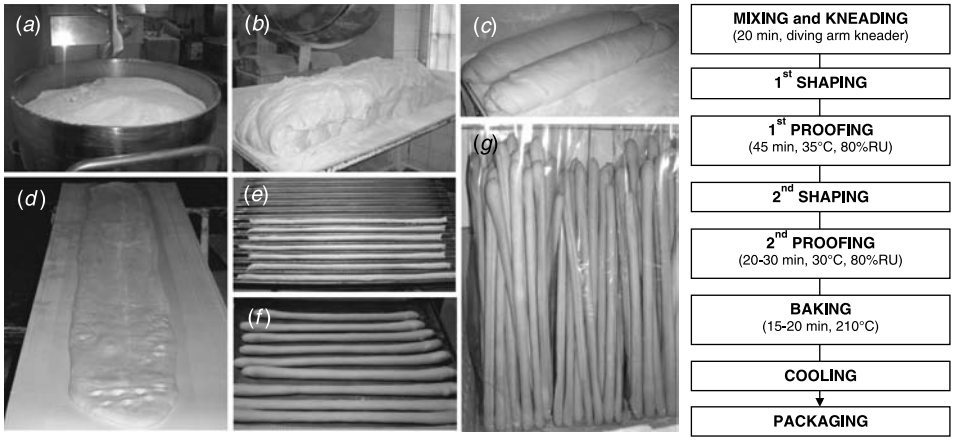


Figure 17.29 *The manufacture of grissini stirati: mixing (a); dough appearance after kneading (b); first shaping and proofing (c); sheeting (d); dough cutting, manual shaping and resting (e); leavening phase (f); packaged product (g). (Courtesy of Panificio Collia, Italy).*

fourteenth century and it was already in wide use in the 1600s (Bordo and Surrasca 2002). The origin of the word is Piedmontese, and derives from *grizia*, a general term for loaf that in time became *gherssin* (small loaf), and hence the modern word *grissino*. A particular category is the *grissini stirati* (stretched *grissini*), which owes its name to the manual shaping technique, which involves stretching and rolling the dough strips by hand after the first leavening phase.

Recognition of the IGP denomination (*Indicazione Geografica Protetta*) for the *Grissino Stirato Torinese* has been initiated to confirm a bond between the product and the Piedmont region. The traditional recipe requires wheat flour, water, yeast, olive oil, and salt. The various phases of the industrial process (Fig. 17.29) include a first shaping of the dough in batches of 5 kg and then a second when the dough is shaped into "tongues" 3 cm thick, 20 cm wide, and 2 m long, and coated with oil. After the first leavening phase at 35°C for 45 min when the dough may be covered with cotton clothes, the "tongues" are cut crosswise into strips 2 cm wide and about 20 cm long. The final shaping of the *grissini* is done manually by holding the rolled strips in the hands and stretching and vibrating the arms until the desired length is obtained. Before baking at 210°C, the dough strips are placed in pans and left to rise again to reach a sufficient alveolar network, which is vital for achieving a highly friable texture.

Other product technologies require the *grissini* to be shaped and cooked in molds. This technique is used for a greater productivity and standardization of the finished industrial product.

Taralli are a typical product of the Puglia region that require the dough to be placed in boiling water before baking (Bordo and Surrasca 2002). It seems that this phase is necessary for impeding the leavening of the product before baking, which in the past took place in a public town bakery. This procedure made it possible to delay baking, unlike bread dough, which must be baked as soon as the leavened dough is ready.

The main ingredients of *taralli* are re-milled semolina, olive oil, water, and salt, to which may be added fennel seeds, compressed yeast, and natural yeast (Table 17.9). The production technology for *taralli* used to be exclusively artisanal. Nowadays, the same procedures are applied while using equipment that ensures greater productivity (Fig. 17.30). Special machinery is used in the industry (*tarallatrici*), which, from a sheet of laminated dough, shapes long rolled strips of dough that are placed inside

TABLE 17.9 ***Taralli*** **Formulation.**

Ingredient	Amount
Main	
Durum wheat re-milled semolina	10 kg
Olive oil	1.5 kg
Water	10% more than farinograph adsorption
Salt	400
Optional	
Fennel seeds	50 g
Compressed yeast	50 g
Natural yeast	0.5–1 kg

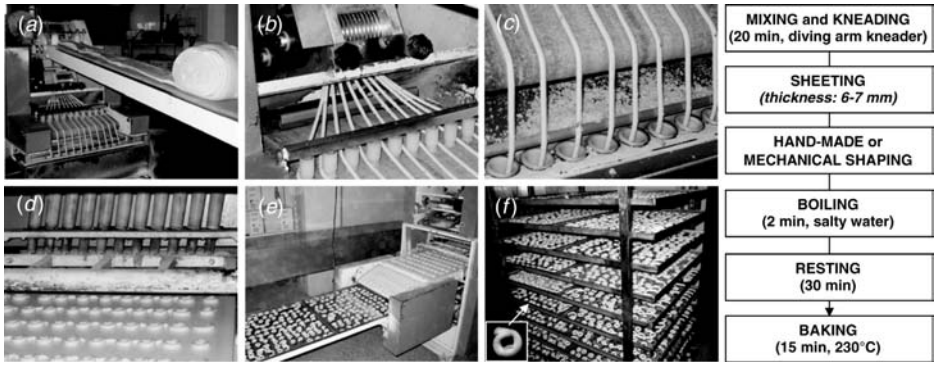


Figure 17.30 *The manufacture of taralli: sheeted dough (a); taralli shaping machine (b); entrance into moving tubes (c); outgoing dough in the characteristic circular shapes (d); taralli entrance into boiling water (e); taralli drying phase before baking in a rotary oven (f). (Courtesy of Panificio Di Gesù, Italy).*

mobile tubes which make a rotating movement. A piece of dough is thus formed into a partially twisted ring (Fig. 17.30*f*) and placed on metal grid trays that are rapidly dipped into boiling water. The trays are then removed, stacked on mobile trolleys, and left at room temperature for 30 min to allow the water absorbed on the surface of the *taralli* to evaporate before baking in a rotating oven.

17.4 CONVENTIONAL AND TYPICAL ITALIAN PRODUCTS FROM CORN

Together with rice and wheat, corn is one of the most widely cultivated cereals in the world. Its production of about 700 million tons is similar in volume to that of wheat, but because of its higher yield it requires a smaller surface area of 60%, thanks mainly to the cultivation of high-production hybrids (Lucisano and Pagani 1997).

Almost 40% of the corn produced in the world is grown in the United States, although European production (EU-25) totals about 53 million tons (Corn Refiners Association 2005). Italy produces approximately 11 million tons of corn, 10% of which is for human consumption, 80% for livestock feed, and 10% for industrial use (D'Egidio 2004).

Compared with other cereal grains, corn is distinctive for its dimensions and the development of the cereal germ that represents approximately 10% of the entire kernel.

Consequently, this cereal, which is rich in lipids, has an important use in the production of a much appreciated seed oil, rich in oleic and linoleic acids.

Corn is often harvested when the grain has high levels of humidity, between 18 and 35%, which prevents long-term preservation. The cereal is therefore subject to drying treatments to reduce the maximum moisture content to about 15%. This is a critical operation, because if it is not performed under suitable conditions the physical properties of the grain could deteriorate, and these properties are important for defining the commercial value of the lot. Too high a temperature, usually greater than 70–80°C, can easily cause stress cracking, with resulting higher fragility of the kernel due to deep fissures.

There are two main processes used for the industrial processing of the kernel (Cantarelli and Lucisano 1983):

1. Wet milling to separate the corn into its chemical parts (starch, proteins, fiber, and oil) by soaking the grains in water (Fig. 17.31);
2. Dry milling to separate the grain into its anatomical parts (endosperm, bran, germ), which are then milled. Flours of different particle sizes are obtained, which are usually reprocessed or, to a lesser extent, used in the production of *polenta* or for ingredients for baked products (Fig. 17.32).

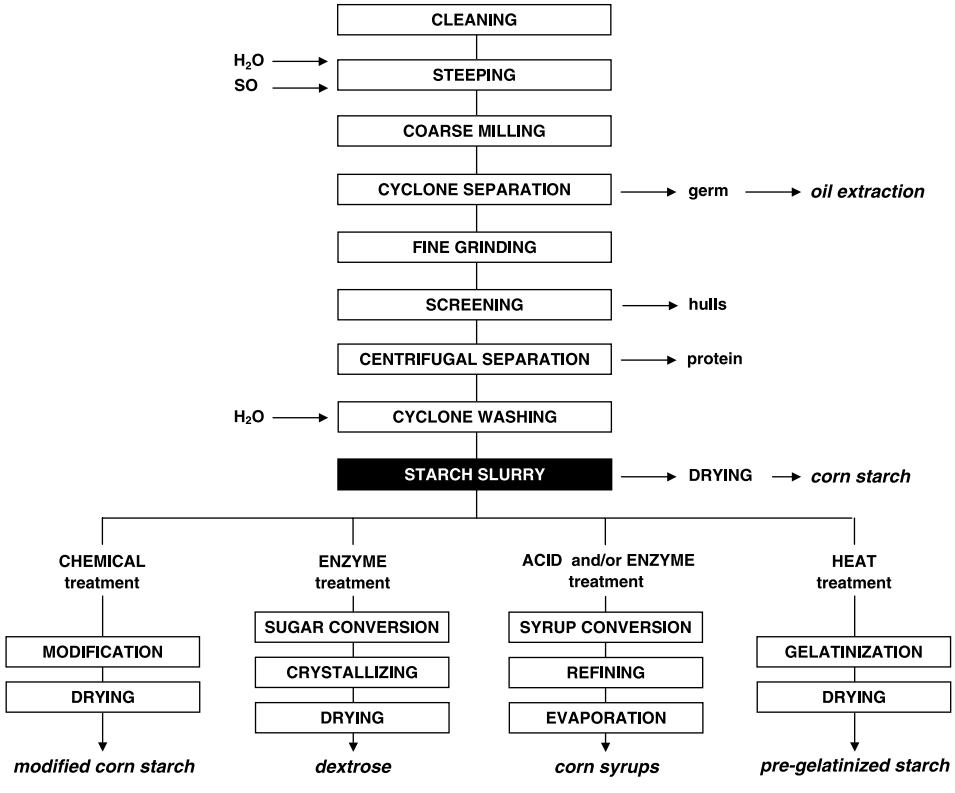


Figure 17.31 *Wet milling of corn.*

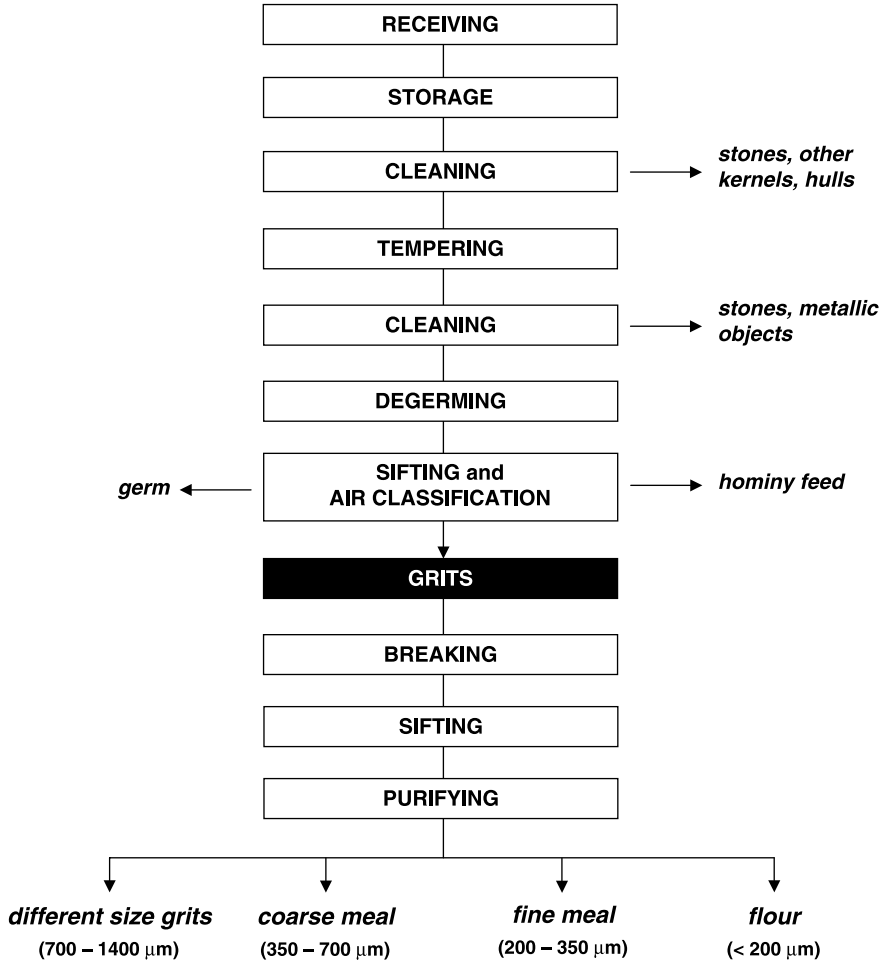


Figure 17.32 *Dry milling of corn.*

17.4.1 Wet Milling

A large part of the world production of corn is destined for use in the starch industry. The starch is extracted from the corn and converted into a large number of byproducts such as modified starches, dextrins, and glucose syrups (Fig. 17.31).

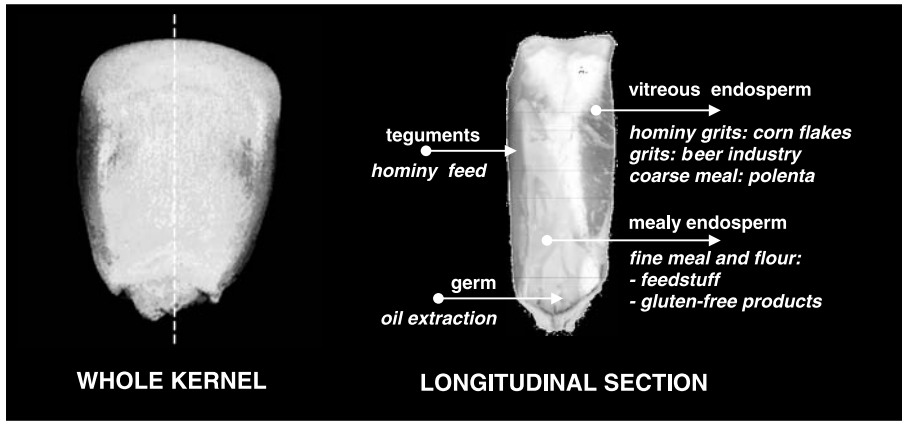
The first operation of the wet-milling process (which gives the name to the entire process) involves wetting the grains by soaking in water that contains small quantities of SO_2 at approximately 50°C for 30–50 h. This compound has antiseptic properties and promotes reduction of protein S–S bridges, facilitating the successive operation of starch separation. The final humidity value of the kernels reaches 45–50%. The endosperm structure is therefore highly softened, facilitating the removal of the cereal germ (the germ is sent on for the extraction of the oil) and the separation of the fibrous and protein materials from the starch during the milling, sifting, and sedimentation phases. The byproducts from this process are widely used in the production of livestock feed (Brockway 2001). The extracted starch is purified and further transformed into an extremely wide range of byproducts, that are widely used as additives in the food

industry: pregelatinized starches, chemically or enzymatically modified starches (thickening and coating agents, and so on), and byproducts of lower molecular weight such as corn syrups. In the latter products, amylose and amylopectin are hydrolyzed into smaller molecules until they become soluble sugars (glucose, maltose, maltotriose, and so on). This process is controlled by choosing the appropriate type of enzyme and appropriate hydrolysis conditions (acidity and time of the treatments). It is therefore possible to produce both high-viscosity syrups with a low sugar concentration and therefore low fermentation levels, as well as syrups with a high sweetening capacity, easily fermentable and with distinct osmotic properties, where the depolymerization of the starch is almost total (Cantarelli and Lucisano 1983).

Further modifications of an enzymatic nature cause the isomerization of the glucose into fructose. The use of this sugar is becoming more and more popular thanks to its sweetening power, which is considerably higher than sucrose.

17.4.2 Dry Milling

The starchy endosperm of corn grain has a peculiar structure: it has translucent vitrous areas, and other opaque and "floury" areas (Fig. 17.33). This difference is due to the different compactness with which the reserve materials, starch, and proteins are stocked during the ripening stage. The percentage distribution between these two areas varies



Corn milling fractions composition

Milling fractions	**moisture** (%)	**starch** (%db)	**protein** (%db)	**lipids** (%db)
Whole kernel	11–15	67–74	8.0–11.5	3.9–6.0
Hominy grits	12.0–14.0	73–75	8.3–8.6	0.7–1.4
Coarse and regular grits	11.5–13.0	81–83	7.4–8.0	0.8–1.8
Coarse meal	11.5–14.0	75–81	6.5–7.5	1.0–2.1
Fine meal	11.5–13.5	77–80	6.5–7.5	2.0–2.5
Flour	11.5–13.5	76–79	6.0–7.0	2.0–3.0
Hominy feed	11–13	67–72	11–13	6.3–7.0
Germ	14–16	50–55	14–15	17–19

%db: percentage dry basis.

Figure 17.33 *View of a whole corn kernel and a section through it. Also, a breakdown of the fractions resulting from milling the corn.*

considerably between each variety. In the most widely grown hybrid in Italy, the dent corn (*Zea mays indentata*), so-called because of the characteristic shape of the kernel, the floury area is in the central part and surrounded by vitrous areas. The kernel of the flint corn (*Zea mays indurata*) is mostly vitreous.

This different structure conditions the destination of the kernel when it is milled with the dry milling process. The vitreous and flint hybrids tend to make up only a small percentage of "flour" (material with particles no greater than a few hundred microns) and are mainly coarsely ground (divided into hominy, coarser grind; and grits, intermediate grind). This material is suitable for transformation into flakes and is highly valued by the beer industry and *polenta* flour producers.

There are two methods that are used for the dry-milling process: milling without removal of the cereal germ, the oldest method, and milling with removal of the cereal germ, the method most used today to ensure a better preservation of the flours (Pomeranz 1987).

The method without removal of the cereal germ grinds the corn grain with a reduced separation of the germ. Flour is obtained by grinding the entire seed with millstones and is aimed at small market niches where its rich and oily flavor is appreciated. Often, the flour is sifted to eliminate the coarser particles of the teguments and the cereal germ. The flour obtained has a lower preservation time with respect to the flours obtained after removal of the germ. This is because the oil, 32–35% of which is contained in the germ, deposits on the surface of the flour particles, increasing the area exposed to oxygen and facilitating the enzymatic attack of the lipases. The different methods available for dry milling with removal of the cereal germ differ greatly in the amount of water added to the grains before the removal of the cereal germ phase (Molino Favero, personal communication). In wet removal of the cereal germ, the corn is brought to a humidity level of 24–25% before milling (this procedure is used for the production of hominy grits for corn flakes). Semiwet processing (corn humidity of about 20%) is used for the production of grits for beer making and in dry milling the humidity level reaches about 15% during the conditioning phase.

Most of the mills in Italy use the dry-milling process (Fig. 17.32), because the main product is coarse flour (*farina bramata*) for the production of the typical Italian product, *polenta*.

After an initial cleaning process using a magnetic separator, a stone separator and an air classification system, the grain undergoes a tempering phase until it reaches a humidity level of 14–15%, which renders the teguments less brittle, swells up the seed germs and facilitates their separation in the degerminator. After a rest phase (of variable duration from 2 to 8 h), the corn is sent through a second cleaning process to completely eliminate any stones or pieces of metal. The grains are then broken up in the degerminator, usually an impact mill. The bran is removed by a classifier aspirator, and the germ and the grits are separated by a gravity separator.

The next phase, the grinding down of the grits, is made using corrugated rolls, sifters, and purifiers, similar to those used in the milling of wheat, but the diagram is less complex with fewer stages at the lower end of the process (Fig. 17.34). During these operations, different amounts of products are produced, according to the endosperm vitreosity of the endosperm: grits of different sizes (700–1400 μm) and flours with particles of different sizes including coarse meal flour (350–700 μm), fine meal flour (200–350 μm), and oil-rich flour (~2.5–3.5% oil), with a particle size <200 μm.

17.4.2.1 Polenta. The coarse meal (called *farina bramata*), a sharp-edge product from the vitreous area of the kernel, is used for the preparation of *polenta*, a typical dish of the regions of northern Italy. *Polenta* was a basic food of the northern Italian regions (Piedmont, Lombardy,

Figure 17.34 *Corn milling equipment. (Courtesy of Molino Favero, Italy).*

Veneto) long before Columbus brought corn to Europe. In those days, the poor rural populations made a porridge with emmer wheat, millet, spelt, or chickpeas, which was cooked on a hot stone. In Roman times, *pulmentum* was the staple of Roman legionnaires, who ate it either in a porridge or in a hard cake form. Later, with the introduction of buckwheat into Italy by the Saracens, *polenta* was prepared with this pseudo-cereal and become more appealing to the taste. Buckwheat *polenta* would eventually fall out of favour when corn from the New World arrived in Italy. The new crop was grown primarily in the northern regions where rainfall was abundant and where other cereals had smaller yields (Rebora 2000). It thus became the most economically viable product grown in the area – the food of the poor peasants. Corn *polenta* was to solve the food problems of the poor until the mid-eighteenth century, with the outbreak of the disease pellagra, caused by a lack of integration of other foods into the diet. Pellagra is caused by a deficiency of the vitamin B group, and especially niacin. The addition of simple supplements such as cheese or other traditional foods such as sausages, fish, or meat make this a versatile and nutritionally complete dish.

Most *polenta* in Italy is nowadays made from corn, and ranges in color from golden yellow to white, according to the variety used. In some areas of the Italian peninsula buckwheat flour mixed with cornmeal is still used for preparing typical local dishes such as *polenta taragna*.

Traditionally *polenta* is cooked in a round-bottom copper pot known as *paiolo*, where water is heated and cornmeal sprinkled into it (Fig. 17.35). The mash is vigorously stirred

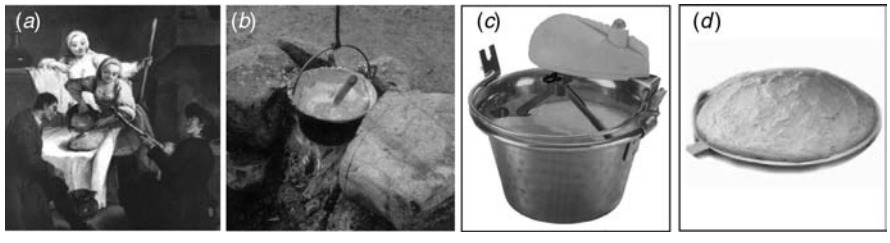


Figure 17.35 *"LA POLENTA" painting by Pietro Longhi (1702–1785) (a); traditional methods of cooking polenta (b, c); presentation of polenta on a wooden board (d).*

for up to 50 minutes with a long wooden spoon and is then poured onto a wooden board and traditionally cut with a piece of string. The long cooking time in water causes the complete gelatinization of the starch and the formation of a more or less compact texture according to the water–flour ratio used.

The long preparation times of this dish can be reduced to just a few minutes if the material is gelatinized in advance at the milling factory. For this purpose, coarse grits are humidified at 18–20% water content and cooked for 35–45 min at 110–125°C under pressure (0.2–0.35 MPa). The material is then rolled out to obtain flakes of thickness 400–500 μm and then dried to a humidity level of 12–13%. There follows the milling and classification phases from which are obtained the precooked *bramata* flour and a small portion of precooked, fine flour.

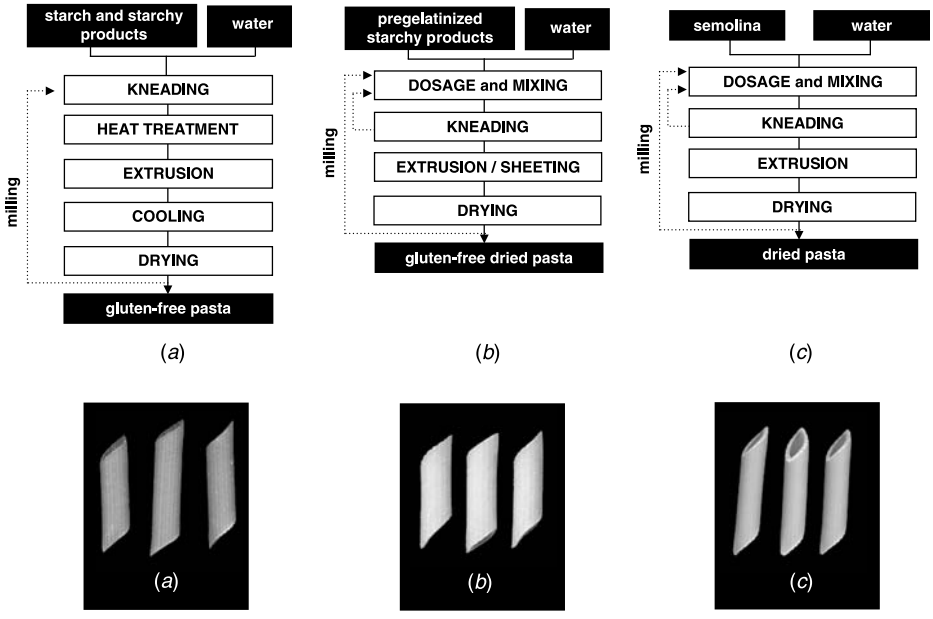
Another type of *polenta* is a ready-to-eat product obtained by cooking the coarse corn flour with water in the presence of an acid solution (citric or tartaric acid) in order to reduce the pH to 3.0–3.5. This consequently increases the product shelf-life, then cooling and packaging, usually in a plastic film. This product can be warmed inside the package or cut and grilled.

17.4.2.2 Corn Flour as Raw Material for Gluten-Free Pasta. During the dry-milling stage of the corn, a small amount (a yield of about 10%) of fine meal or dusted meal is also obtained (Molino Favero srl, personal communication). This product has a starch content that is similar to the one in the coarser grains, a slightly lower protein content, and a higher lipid content of 0.5% (Fig. 17.33). This small amount of byproduct is usually destined to become livestock feed, but is being considered as an interesting raw material for the production of gluten-free products such as bread and pasta, especially if derived from the milling of white corn. These varieties are not only characterized by their neutral color, but also by a more delicate flavor, thus being more suitable for the preparation of products that are traditionally prepared with wheat flour. Precooked meal after size reduction can also be used in the industrial production of gluten-free pasta.

In these types of pasta where the three-dimensional network of proteins is missing, an optimal texture both before and after cooking is assured by the retrograded starch induced by a particular technological process (Fig. 17.36*a*). In fact, a flour that contains starch in its natural form is not capable of forming a compact structure via hydration, mixing, and compacting in the extrusion screw (Resmini and Pagani 1983). A heat-kneading phase that encourages gelatinization of the starch is therefore indispensable. In this way, an easily shapeable mass is formed whose structure is consolidated in the cooling of the product that induces retrogradation of the starch. This procedure, which has been adopted for centuries in the production of oriental pasta (Serventi and Sabban 2000), creates a three-dimensional network where most of the heat-soluble components are restrained in the cooked pasta (Pagani and others 1981). On the contrary, the use of pregelatinized starches or flours makes it possible to use similar technology to that used in the preparation of traditional semolina pasta, thanks to the changes to the starch caused by the previous heat treatment (Fig. 17.36*b*,*c*). In this case the pasta absorbs less water but is subjected to greater cooking loss in the boiling water.

17.5 TYPICAL AND NEW ITALIAN PRODUCTS FROM BUCKWHEAT

Buckwheat is an annual dicotyledon plant that can reach a height of 1 m. A characteristic peculiar to buckwheat is its extremely brief life cycle (60–90 days), which makes it suitable for cultivation at high altitudes as a second crop after the winter crop, and makes it



Cooked pasta quality indices	(a)	(b)	(c)
Weight increase (g/100g pasta)	78.02	57.39	97.43
Solid loss (g/100g pasta)	3.24	7.27	3.76

Figure 17.36 *Pasta production from untreated corn flour (a), pregelatinized corn flour (b), wheat semolina (c), and cooking characteristics.*

highly competitive compared with other kinds of vegetation. The main product of buckwheat is the grain, botanically named achene.

For many centuries buckwheat represented an important source of food for the populations of the Italian Alps and to a lesser extent to the populations of the Apennines (Borghi 1997). Buckwheat is thought to have originated from Manchuria and Siberia and there is evidence that it has been grown in China since 1000 B.C. It is an important crop in Japan, and reached Europe via Turkey and Russia during the fourteenth and fifteenth centuries (Mazza 2000). Today, buckwheat is still used in a number of typical dishes of eastern Europe and Asia such bagels, soba, and noodles (Fig. 17.37).

There are several species of buckwheat, but only the *Fagopyrum esculentum* Moench (common buckwheat) is cultivated worldwide. The production of buckwheat reached its peak at the beginning of the nineteenth century and has been gradually decreasing until today.

The major producer of buckwheat today is Russia, followed by China (Si-Quan-Li and Zhang 2001). With regard to Italy, until just a few decades ago, buckwheat was the most important crop in the mountainous areas of the Alps, in particular the Valtellina area, a valley in the north of Lombardy bordering with Switzerland. However, the period following the Second World War saw a drastic decrease in the cultivation of buckwheat and it has not been grown in these areas for several decades, not even at the smallholder level. Most of the buckwheat consumed in Italy is imported from China as grains. The flour ground in the mills in the mountainous areas of northern Italy is used for the production of

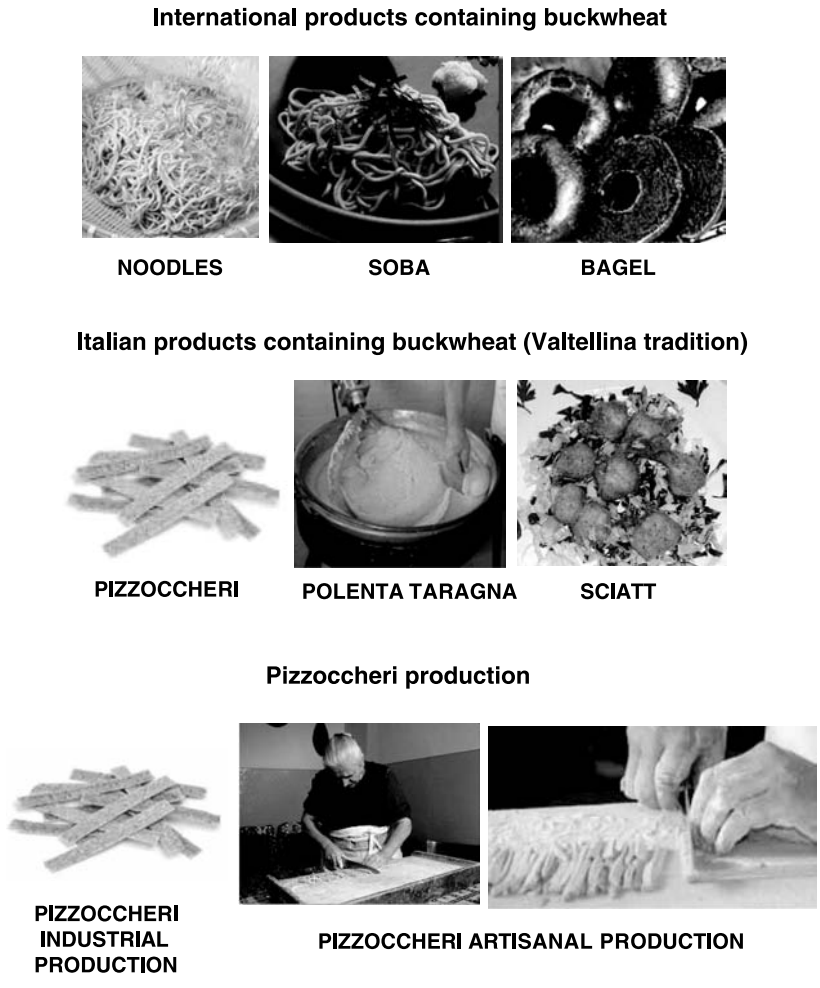


Figure 17.37 *Buckwheat products.*

pizzoccheri or other types of pasta obtained from a mixture of durum wheat semolina and buckwheat flour, and also for the preparation of a dark *polenta* (*polenta taragna*), cheese fritters (*sciatt*) and typical cakes of the area (Fig. 17.37).

The structure of buckwheat is characterized by a very compact hull and by the presence of an embryo with a characteristic sinusoidal shape, which extends throughout the whole cross-section of the grain (Fig. 17.38). The dominant component is starch and its concentration varies from 59 to 70% db (Zhang and Wel 1995). The starch granules of buckwheat are very small (1–7 μm in diameter), clearly grouped in regular clusters delineated by the presence of an air space between them, and their surface is highly irregular in form (Mariotti and others 2006a).

The protein level in the grains can vary between 7 and 21% according to the variety and environmental factors (Biacs and others 2002). Buckwheat proteins present a high biological value, because they are particularly rich in lysine. The proteins, however, are not easily digestible because of the high fiber content, which does not allow proteolytic enzymes easy access to them. The buckwheat achenes contain between 1.5 and 2.7% lipids, but because they are mostly concentrated in the germ the flour contains a much less significant

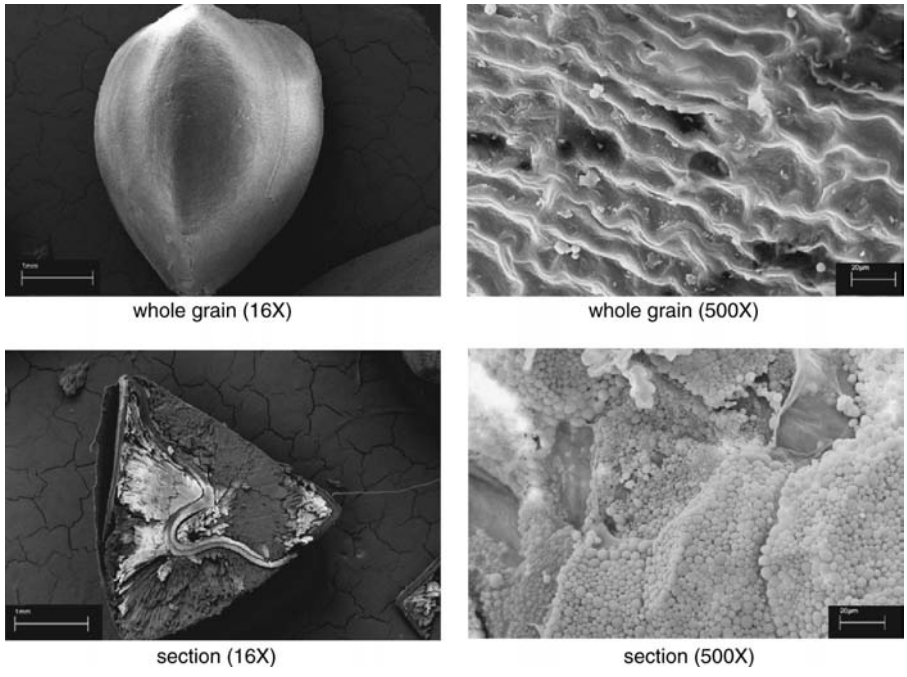


Figure 17.38 *SEM images of buckwheat grain.*

amount. The fatty acids are mainly represented by palmitic acid, oleic acid, linoleic acid, stearic acid, and linoleic acid (Fornal and others 1987).

With regard to the phenolic compounds, the classes mainly represented are the flavonoids, phenolic acids, and condensed tannins. Among the flavonols, rutin is the most important. Rutin is a compound known for its peculiar ability to contrast capillary fragility associated with hemorrhage- or hypertension-related diseases (Kitabayashi and others 1995). Rutin is also effective in reducing cholesterol levels in the blood (Edwardson 1996). Last but not least important is the high content of vitamins, especially B_1, B_2, and E. Buckwheat is also rich in minerals and microelements. The potassium content is similar to that in wheat and there is also a moderate amount of calcium and magnesium. With regard to the microelements, buckwheat contains large quantities of copper and iron.

Buckwheat flour is obtained by grinding the achenes. This is usually performed in one single operation with rolls or millstones. The grain is conditioned to reach a low humidity level, ground until the desired particle size is reached and then sifted. The extraction rates vary between 73 and 78%. Different fractions featuring different content levels of proteins, starch, and ash are obtained (Table 17.10).

TABLE 17.10 Composition of Buckwheat Seed and Milling Fractions.

Component	Seed	De-hulled Seed	Semi-dark Flour	Light Flour	Hulls	Bran
Humidity (%)	12.46	12.55	11.70	12.00	9.75	12.00
Protein (%db)	12.01	13.48	13.81	7.14	3.19	25.19
Starch (%db)	57.17	75.69	71.36	86.49	0.00	52.87
Ash (%db)	2.26	1.80	2.21	0.85	2.31	3.99
Rutin (mg/100 g)	21.70	9.90	22.10	5.00	50.60	15.80

%db: percentage dry basis.

Despite the fact that buckwheat has not been cultivated in Italy for decades, it is still deeply rooted in the culinary traditions of the Alpine valleys, where buckwheat is widely used in the preparation of a large number of local dishes. There is a sector of small commercial enterprises that includes millers, pasta-makers, and factories that use buckwheat grains or flours for the production of pasta, cakes, and other foods. A sector that has not yet been fully developed but which has considerable potential is the health-food segment.

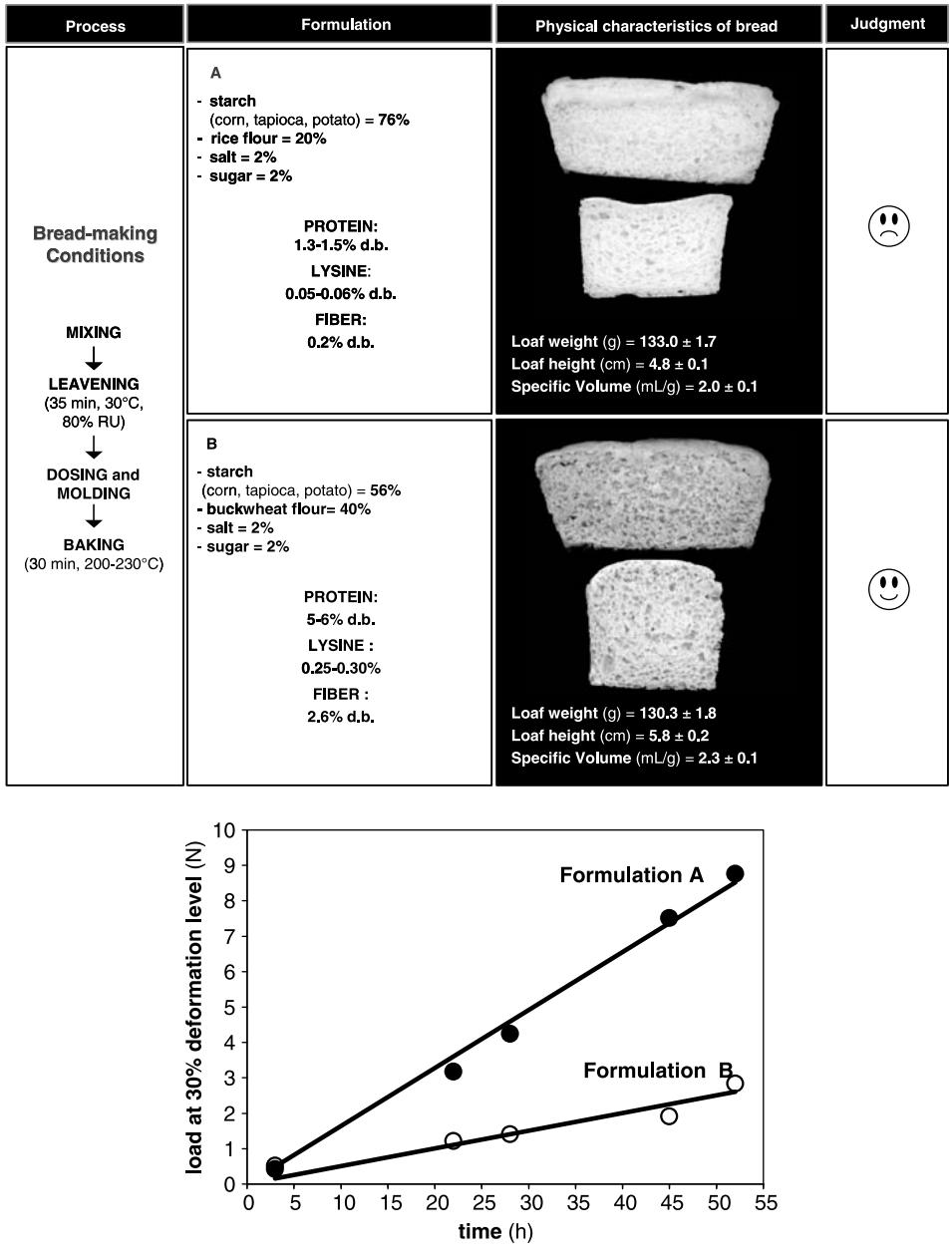


Figure 17.39 *Formulation and physical characteristics of buckwheat-flour bread.*

Buckwheat contains a type of starch that is slower to digest, and so is especially suitable for diabetics (Edwardson 1996). It has recently been observed that buckwheat is fully tolerated by sufferers of celiac disease, and so flour made from buckwheat can be used to produce pasta and baked products.

To this end, the addition of buckwheat flour in a commercial product for the production of gluten-free bread that contains rice flour and starches from various sources has proved interesting. The total substitution of commercial rice flour and starch, each with a concentration of 20% (for a total buckwheat content of 40%), has increased the specific volume of the bread from 2.0 to 2.3 mL/g. This has significantly improved the nutritional value of the formulation, thanks to the protein, lysine, and fiber content (Fig. 17.39). It is important to stress that for this line of products the specific volume does not reach the high values of breads made exclusively of wheat due to the lack of the structural effect of gluten and the lower retention of gas during leavening. A further positive result is the ability of buckwheat flour to maintain the original crumb softness of bread. If, on the one hand, the presence of fiber enables the bread to retain greater quantities of water and therefore improve the softness of the product, on the other it has a dilution effect on the starch present in the original formulation. This results in a lower incidence of starch retrogradation and consequently slower staling kinetics (Fig. 17.39).

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18

Flavor Migration in Solid Food Matrices

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18.1 Introduction 390
18.2 System Development 391
18.2.1 Apparatus for On-Line Measurement of Flavor Concentration 391
18.2.2 A Model Flavor-Dough System 392
18.2.3 Thermal Stability of Flavor Compounds 392
18.2.4 Isothermal Heating System 393
18.3 Methodology for On-Line Flavor Concentration Measurement 393
18.3.1 Photoionization Detection (PID) Method 393
18.3.2 Cold-Trap, On-Line Sampling Method 396
18.4 Quantifying Flavor Migration from Dough 400
18.4.1 Concentration Dependence of Flavor Migration in Formulated Dough 400
18.4.2 Temperature Dependence of Flavor Migration in Formulated Dough 405
18.5 Microwave Heating of Flavor-Formulated Dough 409
18.5.1 Lack of Crispness (Texture) and Browning (Color, Flavor) of Microwave Foods 409
18.5.2 Evaporative Cooling and Steam Distillation 410
18.5.3 A Case Study: Migration of Limonene in Formulated Dough Undergoing Microwave Reheating 411

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18.1 INTRODUCTION

A major problem associated with many flavor systems during heating and baking is loss of desired flavor, and the flavors already present may need to be supplemented. There are several alternative ways to overcome flavor loss due to heating or baking. The most direct and effective is to add excessive amounts of flavor compounds into food matrices. Retention of a flavor compound in a food product cannot be predicted accurately based only on the physical and chemical properties of individual flavor components. There are several important parameters affecting retention of flavor compounds during heating:

1. Temperature,
2. Moisture content of the food,
3. Porosity of the food solid matrix,
4. Molecular weight and volatility of flavor compounds, and
5. Interactions between the food solid matrix and flavor compounds.

To quantify the amount of flavor to add to a product, it is essential to understand the interaction of flavor components with food systems and the parameters that affect flavor migration during heating or baking. Lack of information on migration or breakdown of desirable flavors in food products during heating or baking is also a major concern to the food industry as it formulates products with maximum consumer acceptance for flavor. There is comparatively little available literature on flavor migration in food systems during heating and baking (Thijssen and Rulkens 1968; Solms 1986; Van Osnabrugge 1988, 1989; Karel 1990; Bruin 1992; Eijk 1994; Etzel 1994; Roos and Graf 1995; Hills and Harrison 1995; Voilley 1995; Harrison and Hill 1997; Harrison 1998; Harrison and others 1997, 1998; Hao and others 1999; Springett and others 1999; Hahon and others 2000).

However, in order to obtain useful and meaningful information on the contributions of rates of flavor migration and kinetics of degradation under various conditions, there are several important steps that have to be followed. A unique apparatus for on-line flavor concentration measurement should be developed. For the purpose of measuring flavor migration kinetics in porous solid food systems, an isothermal temperature control for kinetic study had to be constructed that could provide a uniform temperature distribution in a dough sample. Because loss of desired flavor during heating or baking can also be attributed to thermal degradation of flavor compounds, a thermally stable compound is needed. If a compound is not thermally stable then total flavor loss will be contributed to by both thermal degradation and migration. So, a thermally stable flavor compound is needed to demonstrate the usefulness of this apparatus for studying flavor migration kinetics.

This chapter is organized according to a research project on moisture migration and flavor migration in solid food matrices (Fu 1996). The following sections will attempt to provide an understanding of the heat and mass transfer processes of flavor compounds based on experimental data and mathematical models, as available. Details of the on-line and off-line flavor concentration measurement techniques are also discussed. A final comment is that there is no single effect which explains the rate of migration of flavor. This approach can be adopted as a tool to increase our understanding of parameters that affect flavor migration rate in porous solid food systems.

18.2 SYSTEM DEVELOPMENT

This section will discuss how to design and build a closed vessel for holding the flavor-dough sample, which provides a minimal system response time for on-line measurement of flavor concentration. This section will also explain how to formulate a model flavor-dough system and to fulfil isothermal heating condition in the sample at desired temperatures by applying microwave energy to achieve isothermal conditions.

18.2.1 Apparatus for On-Line Measurement of Flavor Concentration

A plastic vessel (Fig. 18.1) with an appropriate geometry and venturi angle-taper was designed for holding samples. Two cone-shaped plastic bases with an angle of 14° were used to minimize air turbulence around the sample and also to reduce inside vessel volume to minimize system response time. Carrier gas is forced through a fitted plastic disk to achieve an even distribution. Either solid food or liquid materials, such as flavor or oil, may be placed in the vessel. The vessel was made from acetal resins, which are resistant to a wide variety of chemicals and have a maximum recommended continuous use temperature in air of 105°C (Sinker 1986). The mean residence time of carrier gas at flow rates of 243, 348, and 595 mL/min are 7, 6.25, and 5.5 s, respectively. A carrier gas flow rate of

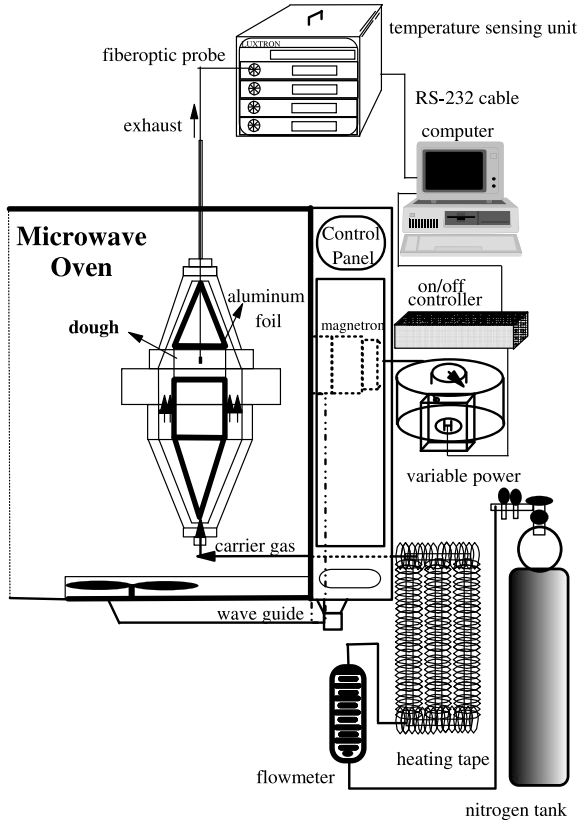

Figure 18.1 *Schematic diagram of the flavor on-line experiment. (Fu and others 2001).*

595 mL/min was selected, because it offers the smallest mean residence time that is insignificant compared to the 1- to 5-h heating times used in the experiments (Fu and others 2003a).

18.2.2 A Model Flavor-Dough System

Dough matrices and flavor compounds have been separated, ideally, into two kinds based on hydrophobicity (Lund 1993). Hydrophobic and Hydrophilic model food matrices were selected, as were hydrophobic and hydrophilic flavor compounds having similar volatility and molecular weight. A hydrophilic flavor (ethyl acetoacetate) and three hydrophobic flavors (limonene, *tert*-butylbenzene, and 2,5-dimethylpyrazine) were chosen. Ethyl acetoacetate, limonene, and *tert*-butylbenzene were chosen because they have similar boiling points (181, 176, and 169°C, respectively) and similar molecular weights (130, 136, and 134, respectively), allowing us to minimize the effects of these parameters in the interpretation of the data. 2,5-Dimethylpyrazine (boiling point 108°C, molecular weight 155) is a thermally induced product of the Maillard reaction and is an important flavor compound for roasted and toasted foods. The flavor compound must be thermally stable during heating and baking. Gelatinized flour-based and gluten-based systems were chosen to serve as hydrophilic and hydrophobic systems, respectively. Regarding porosity distribution, the flour-based hydrophilic dough had a very even texture and uniform porosity distribution. However, the gluten-based hydrophobic dough had a wide porosity distribution, with large air pockets and areas of high dough density. Based on the above analysis and observations, the best model dough system was a flour-based dough system with moisture content between 40 and 50% and porosity between 0.5 and 0.6 (Fu and others 2003a).

18.2.3 Thermal Stability of Flavor Compounds

Flavor compounds selected for study must exhibit thermal stability under three conditions:

1. Thermal stability in the absence of dough;
2. Thermal stability in the presence of uncooked dough; and
3. Thermal stability in the presence of dough that is being heated.

Recovery efficiency and accuracy of the isolation procedure need to be determined before proceeding to reaction experiments. The results show that the four abovementioned flavors are thermally stable in the absence of dough when exposed to steam and when heated with dough in which the starch has been gelatinized (cooked) (Fu and others 2003a). Thermal stability of flavor compounds in flour-based matrices was assessed in a flavor material balance experiment. The experiment was conducted by adding known amounts of flavors into the flavor-dough sample, mixing well, and heating the flavor-mixed raw dough with steam for varying amounts of time. The results show that the three flavor compounds 2,5-dimethylpyrazine, *tert*-butylbenzene, and limonene were thermally stable when mixed with all flour ingredients and steam-heated. However, the more severe the heat treatment, the less ethyl acetoacetate was extracted from the matrices. Compounds indicating chemical degradation were not detected by gas chromatography (GC) analysis. A possible explanation is that ethyl acetoacetate chemically reacted with starch during the heat processing, resulting in poor extraction. Consequently, ethyl acetoacetate is either not stable or it irreversibly binds with ingredients when steam heated (Fu and others 2003a).

From this experiment, in order to totally extract the flavor from the matrices, the particle size of the flavor-formulated dough must be very small and the extraction time needs to be very long. The flavor-dough matrices must therefore be ground or pressed to reduce particle size before applying further extraction processes.

18.2.4 Isothermal Heating System

The microwave system consisted of a modified microwave oven, a fiber-optic temperature sensing system, an on/off temperature controller, and a computer (Fig. 18.1). A microwave oven rated at 700 W was modified so that the field intensity in the cavity could be changed continuously. A detailed description of this system can be found in Tong and others (1993). The optical-fiber temperature probe was centered in the sample. When a program was started, upper and lower set-point temperatures, $\pm 0.1^{\circ}C$ of the desired set-point isothermal temperature, were input by a computer program for data acquisition and control. Eight fiber-optic temperature probes were inserted to map the sample temperatures. With accurately controlled microwave energy input and preheated carrier gas, the sample was maintained at the desired temperature without significant temperature profiles. For the constant-temperature experiments, the average temperature and temperature variation of flour-dough samples with porosity 0.42 and initial moisture content of 0.48 wet basis (w.b.) were $38 \pm 0.8^{\circ}C$, $57 \pm 1.4^{\circ}C$, $76 \pm 1.7^{\circ}C$, $85.5 \pm 1.9^{\circ}C$, and $90 \pm 2.2^{\circ}C$ through the heating and accompanying drying processes (Fu and others 2003a). Temperature variance was not dependent on the location of the control probe. Temperature gradient deviation can be further reduced if the sample size is reduced or porosity is increased.

18.3 METHODOLOGY FOR ON-LINE FLAVOR CONCENTRATION MEASUREMENT

The methodology for measuring on-line flavor concentration in effluent gas from a closed vessel is discussed in this section. A photoionization detection method (PID) and two cold-trap on-line methods based on an isolation technique will be discussed.

18.3.1 Photoionization Detection (PID) Method

Photoionization has been used as a means of detection for about 25 years. The photoionization detector is designed to detect the concentration of gases present in the effluent from a gas chromatograph, utilizing the principle of photoionization. Photoionization occurs when a molecule absorbs a photon of sufficient energy to cause an electron to leave its orbit and create a positive ion. If the ionization potential of the compound is less than the lamp energy, then the PID will respond to that molecule (Driscoll 1977). Compared to the flame ionization detector, the PID has an enhanced response for aromatic compounds. This improved selectivity allows the instrument to operate as either a continuous or chromatographic analyzer, because the PID does not respond to light hydrocarbons such as methane (Driscoll and others 1982). Further, as a result of its versatility and performance, PID is one of the most widely used selective detectors for GC (Driscoll 1985). By utilizing UV lamps with different photon energies, the detector can be modified easily for either almost-universal or quite-selective detection of compounds.

A schematic diagram of the system for on-line measurement of flavor concentration using PID is presented in Figure 18.2. By adjusting the metering valves and vacuum

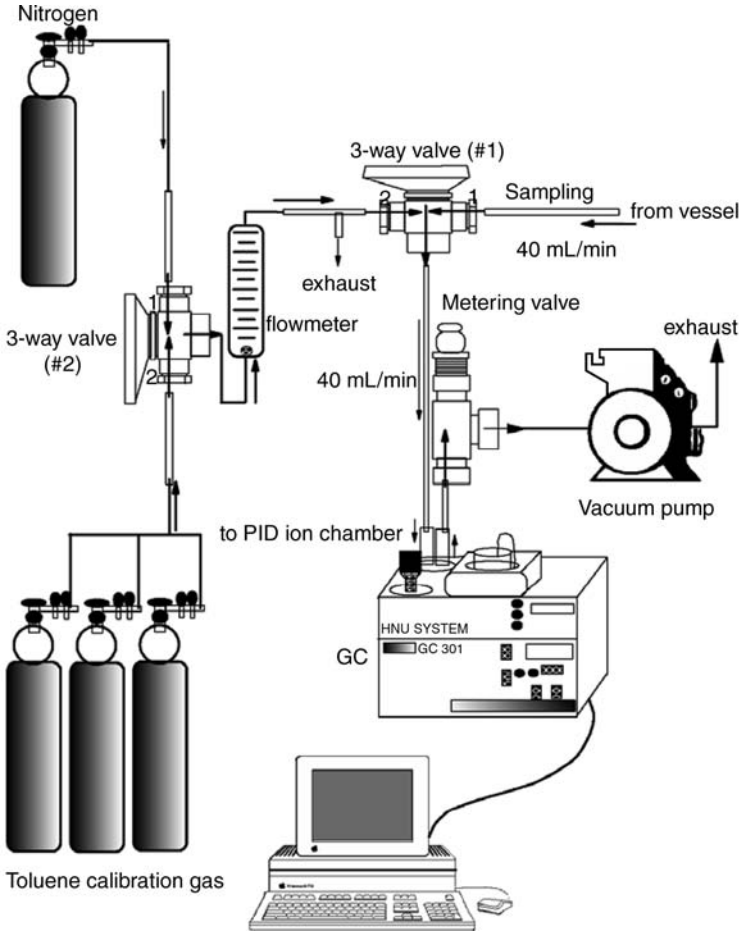

Figure 18.2 *Schematic diagram of the system for on-line measurement of flavor concentration by using PID method. (Fu and others 2001).*

pump, a gas stream (40 mL/min) carrying the flavor may be separated from the main flow, entering directly through the inlet into the ion chamber. No GC column is required for this method. Four tanks of individual gas mixtures (2, 12.7, 22, and 127 ppm toluene in nitrogen) were purchased to calibrate the photoionization detector. One nitrogen tank was used for checking the baseline of the PID detector before and after the experiment. A pair of three-way valves were used to control the gas flow. By changing the first three-way valve to position 1, the system measured flavor on-line. After removing the sample from the vessel, the first valve was immediately changed to position 2, and the second to position 1 (nitrogen flow) to check the baseline. After this, the second valve was changed to position 2 to run the toluene calibration curve using different concentrations of toluene mixtures. Serial dilutions of toluene/2,5-dimethylpyrazine, toluene/ethyl acetoacetate, toluene/limonene, and toluene/*tert*-butylbenzene solutions were prepared in methylene chloride to cover the dynamic range of the instrument. Aliquots of each solution were injected in triplicate, and the average result was used for all future calculations. The full concentration range was studied with the carrier gas flow constant at 40 mL/min. Solution concentrations were converted to micrograms of material injected,

based on an injection volume of 1 μL. Then, values for *peak area* (*response values*)/*weight* were calculated for each compound. In addition, relative responses for limonene and ethyl acetoacetate to toluene were calculated for each solution. The relative response factor, K_f, normalized to toluene on a weight basis, was calculated using the following equation:

$$K_f = \frac{peak\ area_i}{weight_i} \Bigg/ \frac{peak\ area_{toluene}}{weight_{toluene}} \tag{18.1}$$

where K_f is the relative response factor, $weight_i$ is the injected weight of compound i, and $peak\ area_i$ is the area of the GC peak for compound i. Daily calibration was performed using a toluene-gas standard (mixed with nitrogen) passed through the GC detector. Four toluene calibration points (2, 12.7, 22, and 127 ppm) were used to calibrate PID voltage responses at attenuations of 1, 10, and 100. The toluene-gas flow rate through the PID was exactly that used for collection of the gases passing through the vessel. The PID voltage response to the toluene-gas standard, and the PID gas-flow rates through the vessel were recorded, with the measurement protocol very tightly controlled. Toluene-gas concentration is given in volume percent, and can be converted to mass concentration as follows:

$$\frac{Volume_{toluene}}{Volume_{nitrogen}} = \frac{\left(\frac{Weight_{toluene}}{92.14}\right)}{\left(\frac{Weight_{nitrogen}}{28.012}\right)}, \qquad (PV = nRT), \tag{18.2}$$

$$[C]_{toluene}\ (\text{ppm, by weight}) = \frac{g_{toluene}}{g_{nitrogen}} = \frac{l_{toluene}}{l_{nitrogen}}\ (\text{ppm, by volume}) \times 3.2893. \tag{18.3}$$

Concentrations of limonene, ethyl acetoacetate, *tert*-butylbenzene, and 2,5-dimethylpyrazine in the effluent gas from the vessel can be calculated directly from the PID output voltage as follows:

$$\frac{[C]_i}{peak\ area_i} = \frac{[C]_{toluene}}{peak\ area_{toluene}} \Bigg| K_f, \tag{18.4}$$

where $[C]_i$ is the flavor concentration in the effluent gas from the vessel, $(g_{flavor}/g_{nitrogen})$, and

$$[C]_{toluene} = \text{toluene standard gas}, \qquad \frac{g_{toluene}}{g_{nitrogen}}.$$

The daily toluene-calibration curve, $voltage_{toluene}$ *vs.* $[C]_{toluene}$, can be converted to a daily flavor-calibration curve using Equation (18.4). As the GC detector was only used for measuring the concentration of a single flavor compound in effluent gas from the vessel, the sampling gas, at a flow rate of 40 mL/min, should have the same flavor concentration as the main gas stream. The amount of flavor emitted from the sample can be calculated as follows:

$$g\ \text{of flavor} = \left(\int \cdot\, dt\right)(gas\ flow\ rate) \cdot (gas\ density), \tag{18.5}$$

where $(\int \cdot\, dt)$ is the integral area under the *concentration* (ppm) *vs. time* curve.

The following is an example of calculations for on-line measurement of limonene concentration using PID. Daily calibration was carried out by passing a toluene-gas standard (mixed with nitrogen) through the PID detector. Based on the relative response value for limonene, the toluene calibration curve can be transformed to the limonene analog using Equations (18.3) and (18.4). Figure 18.3 presents the results of an on-line experiment to transfer voltage output *vs*. time for the limonene analog using a limonene calibration curve. Using Equation (18.5), we can further transfer the limonene concentration *vs*. time to the cumulative analog (Fig. 18.3). Then the weight loss, as measured by a balance, is compared to the amount of PID-detected flavor. Performance of the on-line apparatus and efficacy of the associated protocol were verified by conducting a series of recovery and retention experiments using four flavor compounds, with excellent observed recoveries ($101.7 \pm 1.5\%$, $n = 13$) (Fu and others 2001). Photoionization detection provides rapid and accurate on-line measurement, with its enhanced and selective response features most suitable for on-line measurement of flavor at lower concentrations.

Several important points need to be emphasized. Using nitrogen as a carrier gas, a negative PID-response peak has been reported for injection of various gases (Driscoll 1977; Senum 1981). Other gases with comparable electron capture, such as nitrous oxide (N_2O) and, to a lesser extent, carbon dioxide (CO_2), exhibit a similar, negative PID response. It is more likely that the greater predominance of the removal mechanism, which results in a reduction in PID background current, was due to quenching via electron capture rather than the quenching mechanism (Senum 1981). The negative PID signal response observed by Fu and others (2001) is possibly due to quenching via electron capture from water and oxygen. Although a reduced quenching effect has been reported for carbon dioxide compared to oxygen, similar investigations have never been undertaken for water. Fu and others (2001) hypothesize that water has a more significant quenching effect than either oxygen or carbon dioxide. In future studies of flavor-concentration measurement from a real, isothermally heated, solid food matrix, water and oxygen should be trapped using a molecular sieve, gas purifier, line filter, and so on, to prevent effects associated with PID quenching via electron capture.

18.3.2 Cold-Trap, On-Line Sampling Method

In the selection of an appropriate isolation technique for on-line measurement of flavor concentration from heated, flavor-formulated dough, requirements to obtain accurate quantification include:

1. Single batch processing;
2. On-line sampling in very small quantities, taking as many samples as possible (for example, 20 samples for one measurement);
3. Measurement of flavor migration at low temperatures (for example, 40°C), requiring sampling in time periods in excess of 5 h;
4. Total desorption, and
5. Accurate quantitative analysis of the compounds of interest.

Two different cold-trap methods, which meet the above requirements, were developed to measure single or multiple flavor compounds in effluent gas from the closed vessel with or without water present (Fu and others 2003a). The principles of these set-ups are the

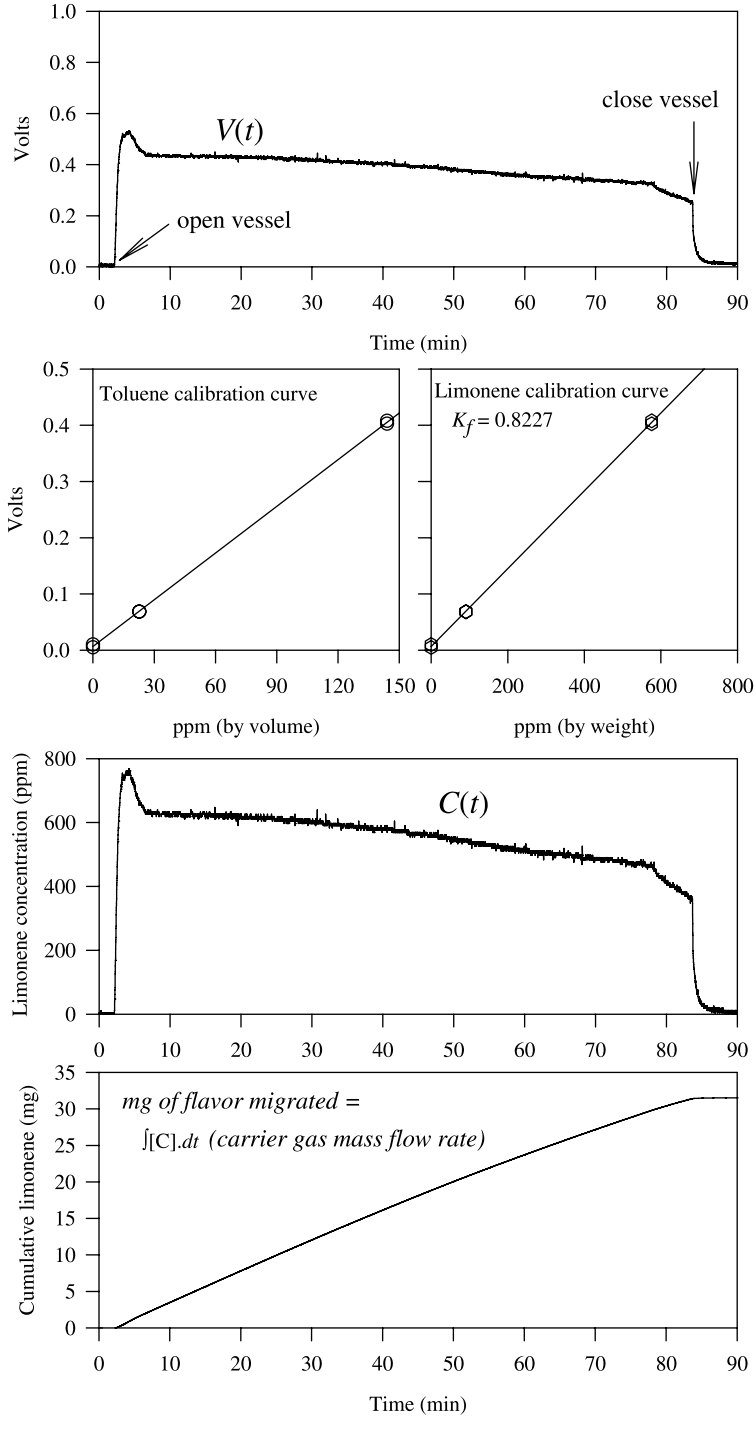

Figure 18.3 *Illustration of transforming voltage output vs. time to cumulative flavor migration vs. time. (Fu and others 2001).*

removal and subsequent liquid absorption of the volatile flavor compounds in the effluent gas from the vessel at very low temperatures. The gas samples are allowed to flow through a solvent in which the volatile compounds are dissolved and cold-trapped at −24 to −36°C.

In the first method (Fig. 18.4), the carrier gas passing around the small container carries the flavor in the headspace out of the vessel into a cold-trap unit. The cold-trap unit consists of a 68-mL glass tube with screw cap (25 × 200 mm) containing 50 mL methylene chloride placed inside a cold bath at −36°C (40% antifreeze and 60% water). A piece of plastic tubing (0.3175 cm, i.d.) is passed through the cap, terminating at the bottom of the tube in a mobile phase filter, which is so designed that a flow of finely dispersed purge gas (nitrogen and volatiles) is dispersed through the solvent, methylene chloride. The carrier-gas flow rate is either 375 mL/min or 645 mL/min. After capturing flavor compound for 2–5 h, the tube was removed and the trapping liquid analyzed for flavor concentration by GC. An aliquot of the trapping solvent was removed from the long tube at different times using a syringe with a 15-cm-long needle and stored in a 0.3-mL-capacity microvial. The amount of sample in the microvial was weighed and equilibrated to 27°C for subsequent GC analysis. Routinely, 10–15 samples of 0.15 g each were obtained for on-line measurement. This procedure worked well for effluent gas containing no water (Fu and others 2003a). For gas effluent containing water vapor, it was necessary to design a system that avoided plugging the inlet tube with ice. The system presented in Figure 18.5 was developed for this purpose (Fu and others 2003a). The glass test tube was replaced by a 100-mL three-necked round-bottomed flask containing 70–90 mL methylene chloride. The diameter

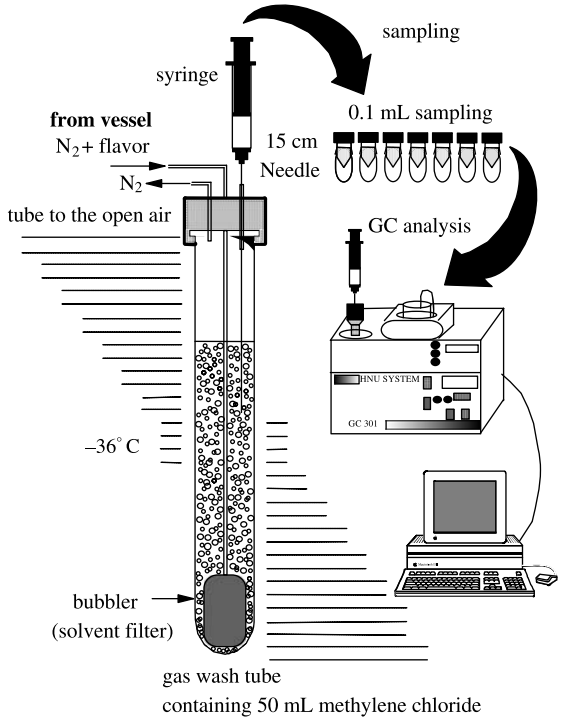

Figure 18.4 *Schematic diagram of the system for on-line measurement of flavor concentration by using cold-trap method with a long tube. (Fu and others 2003a).*

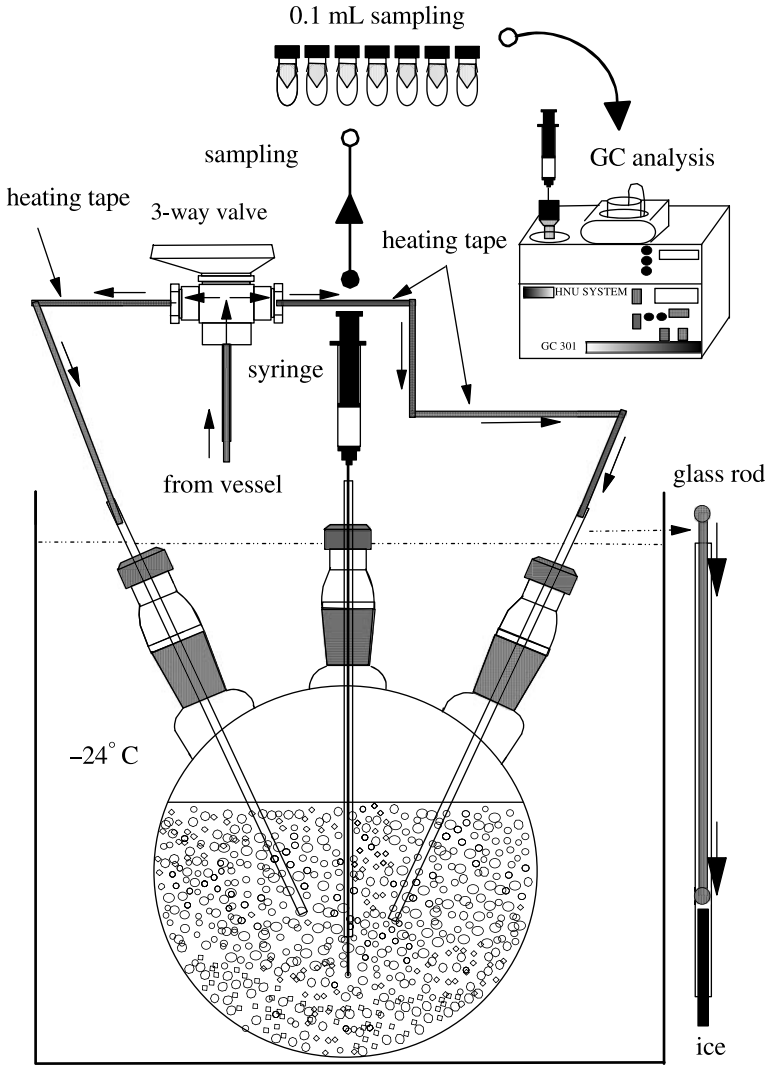

Figure 18.5 *Schematic diagram of the system for on-line measurement of flavor concentration by using the cold-trap method with a three-necked flask. (Fu and others 2003a).*

of plastic tubing was increased from 0.3175 to 0.635 cm. To overcome blockage due to frozen water, a three-way valve was used to switch from one gas effluent supply line to another. A round glass rod (3 × 125 mm) was used to push the frozen ice into the flask and clear the gas passage of the plastic tubing that was not in use. By switching the three-way valve, the system was efficiently controlled without ice blockage. The ice in the solvent was easily separated from the solvent. Heating tapes insulated with fiberglass cloth were installed on all inlet gas lines to the cold-trap unit to prevent condensation. The performance of the cold-trap on-line sampling method was verified by conducting a series of recovery and retention experiments:

1. Recovery of pure flavor compounds, which simulated removal of aroma from food and demonstrated recovering the compound;

2. Retention of pure flavor compounds, which demonstrated the efficacy of capturing flavor compounds of interest; and
3. On-line sampling measurement of pure flavor compounds, which affirmed the procedures for calculating on-line flavor concentration and reconfirmed the suitability of using the cold-trap method for on-line sampling flavor concentration measurement.

This quantitative study for demonstrating a material balance on the flavor compound has been described by Fu and others (2003a). Good quantitative recovery data were obtained. This method can therefore be used for measuring flavor concentration on-line.

18.4 QUANTIFYING FLAVOR MIGRATION FROM DOUGH

A new approach using cold-trap, on-line sampling and off-line methods was applied to investigate the migration rates of limonene, *tert*-butylbenzene, and pyrazine in a flour-dough matrix. The term "off-line flavor concentration measurement" is used to describe the method by which the residual concentration of the flavor material in the sample is measured after the sample is removed from the apparatus and subsequently subjected to solvent extraction to measure its flavor concentration. Instead of measuring residual flavor concentration (off-line method) in the matrix using solvent extraction of the matrix, the term "on-line sampling flavor concentration measurement" is used to describe the method by which the flavor compound that migrates out of the sample into the gas stream is captured and the solvent is subsequently analyzed for flavor concentration.

In the following sections, cold-trap on-line sampling and off-line methods are used to investigate the dependence of the migration of flavor compounds in a real solid food matrix (flour dough) on concentration under different isothermal temperatures. Temperature dependence was appropriately modeled by the Arrhenius relationship. The activation energies for migration of limonene, pyrazine, and water were calculated.

18.4.1 Concentration Dependence of Flavor Migration in Formulated Dough

A majority of reactions occurring in foods obey well-established kinetics. Thermal destruction of microorganisms, most nutrients, quality factors (texture, color, and flavor) and enzymes generally obey first-order reaction kinetics (Lund 1975). The reaction rate constant defines the reaction rate. The reaction rate constant defines the reaction rate. There are several ways in which the rate of a chemical reaction can be reported. To determine reaction kinetics, it is essential to know the extent of the reaction at any time. The extent of reaction at any time or the fractional conversion f at a constant temperature can be described as

$$f = \textit{fraction accomplished} = \frac{\textit{amount of flavor removed}}{\textit{maximum amount that can be removed}}. \tag{18.6}$$

When the flavor concentration as a function of time is known, f can be defined as

$$f = \frac{C_0 - C_t}{C_0 - C_\infty}, \tag{18.7}$$

where C_0 is the initial flavor concentration (g/g dry solid) at time 0, C_t is the flavor concentration (g/g dry solid) at time t, and C_∞ is the equilibrium flavor concentration (g/g dry solid) at ∞. For moisture, the values of the equilibrium moisture content C_∞ are relatively small compared to C_t or C_0, and can be assumed to be zero. However, for flavors, the values of the equilibrium flavor content C_∞ generally may not be assumed zero and can also be temperature-dependent.

The data were analyzed using a first-order reaction kinetic model (first-order rate constant k) with temperature dependence described by the Arrhenius equation (activation energy E_a) and the decimal reduction time model (D-value):

$$-\frac{dC}{dt} = k(C - C_\infty), \qquad \ln\frac{C_t - C_\infty}{C_0 - C_\infty} = \ln(1-f) = -kt; \tag{18.8}$$

$$-\frac{dC}{dt} = \frac{\ln(10)}{D}(C - C_\infty), \qquad \log\frac{C_t - C_\infty}{C_0 - C_\infty} = \log(1-f) = -\frac{t}{D}; \tag{18.9}$$

$$\frac{1}{D} = \frac{k}{\ln(10)}, \qquad D = \frac{\ln(10)}{k}; \tag{18.10}$$

$$\ln k = \ln A_0 - \frac{E_a}{RT}; \tag{18.11}$$

where A_0 is the pre-exponential constant, E_a is the activation energy (J/mol), R is the universal gas constant (8.314 J/mol), and T is the temperature in kelvin (K). The activation energy expressed in terms of k is given by

$$\frac{k_2}{k_1} = \exp\left[-\frac{E_a}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)\right]. \tag{18.12}$$

Regardless of isothermal temperature, the flavor-formulated dough samples were heated sufficiently long for the moisture content to approach the equilibrium moisture content (close to zero). As shown in Figure 18.6, moisture content decreased exponentially ($r^2 \geq 0.99$) with time at each temperature for each flavor-formulated sample (Fu and others 2003d). Pyrazine's behavior appeared very similar to that of water. Because pyrazine is more water soluble than either limonene or *tert*-butylbenzene, it is expected that pyrazine would be vapor-stripped from the dough in some direct proportion to water vapor. An important observation of the behavior of the flavor concentration is that the flavor concentration of limonene, *tert*-butylbenzene, and pyrazine dropped precipitously when moisture content approached 0.1 g water/g solid and subsequently remained unchanged (Fu and others 2003b). An explanation for the abrupt change in flavor concentration at a moisture content around 0.1 g water/g solid is flavor encapsulation (Karel 1990; Reneccius 1990). Thus, due to changes of the matrix structure during drying, flavor compounds may be physically entrapped, presenting a difficulty for extraction by methylene chloride. Consequently, data from the off-line method may not represent the true flavor concentration.

Figure 18.7 presents limonene concentration in formulated dough as a function of time at 85.5°C for varying initial limonene concentrations (Fu and others 2003b). The largest concentration (0.08 g/g solid) from on-line experiments is about twice that from off-line experiments (0.035 g/g solid). Flavor concentration decreased with time and leveled off after about 50 min heating. Figure 18.7 also presents data on limonene concentration as a function of moisture content for both on-line sampling and off-line

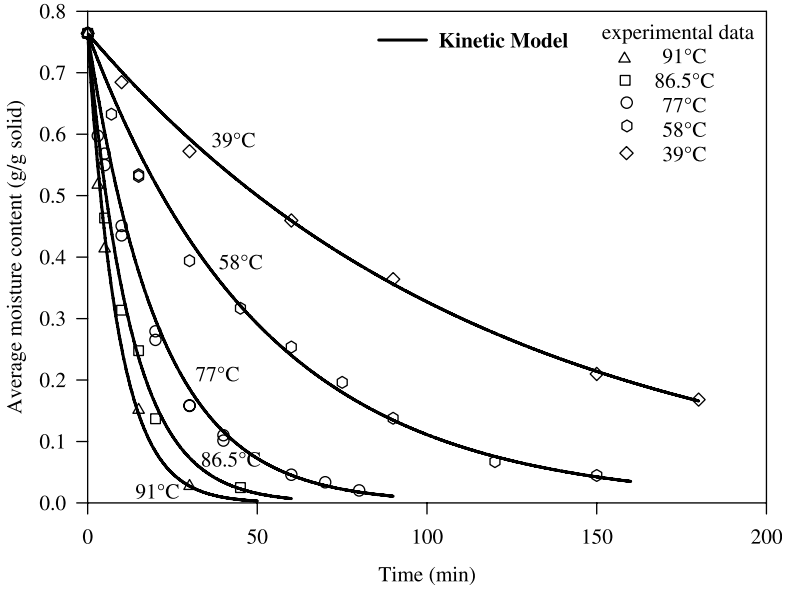

Figure 18.6 *Moisture content-time curves for dough during isothermal heating at various temperatures (porosity = 0.55, initial moisture content = 0.764 g/g solid). (Fu and others 2003d).*

measurements during isothermal heating at 85.5°C. It can be clearly seen that the data from off-line measurement did not present the true concentration of limonene left in the dough matrix at moisture contents less than about 0.1 g water/g solid. If moisture content is greater than 0.1 g water/g solid, the flavor concentration measured from off-line experiments was about the same as that measured by on-line sampling. At higher moisture content, the solvent extraction method efficiently extracts the limonene from the matrix. However, methylene chloride is not capable of extracting all limonene from the matrix when moisture content is less than around 0.1 g water/g solid. As described earlier, this is due to physical entrapment of flavor at lower moisture contents.

Several important points need to be emphasized. In preliminary studies (Fu and others 2003a), it was determined that an extraction time of 48–72 h at room temperature was necessary to completely extract limonene, *tert*-butylbenzene, pyrazine, 2,5-dimethylpyrazine, and ethyl acetoacetate from the dough matrix. It was reasoned that diffusion limited extraction of the flavor compounds by the nonpolar solvent from the hydrophilic matrix. However, at moisture content lower than 0.1 g water/g solid, an even greater effort was necessary to extract limonene from the samples. In fact, the flavor was never completely extracted from the matrix by methylene chloride. Several experiments were also conducted in which the rehydrated, ground-dried samples were heated in a sealed ampule by steam at 100°C for an additional two hours followed by extraction by methylene chloride. An additional 10% was recovered from the matrix. Although it may be possible to find a solvent that can infiltrate the encapsulated structure and extract the limonene, that was not the purpose of this study.

Under this isothermal heating condition, the equilibrium concentration of limonene is predictable. Equation (18.13) relates the equilibrium concentration C_∞ to initial

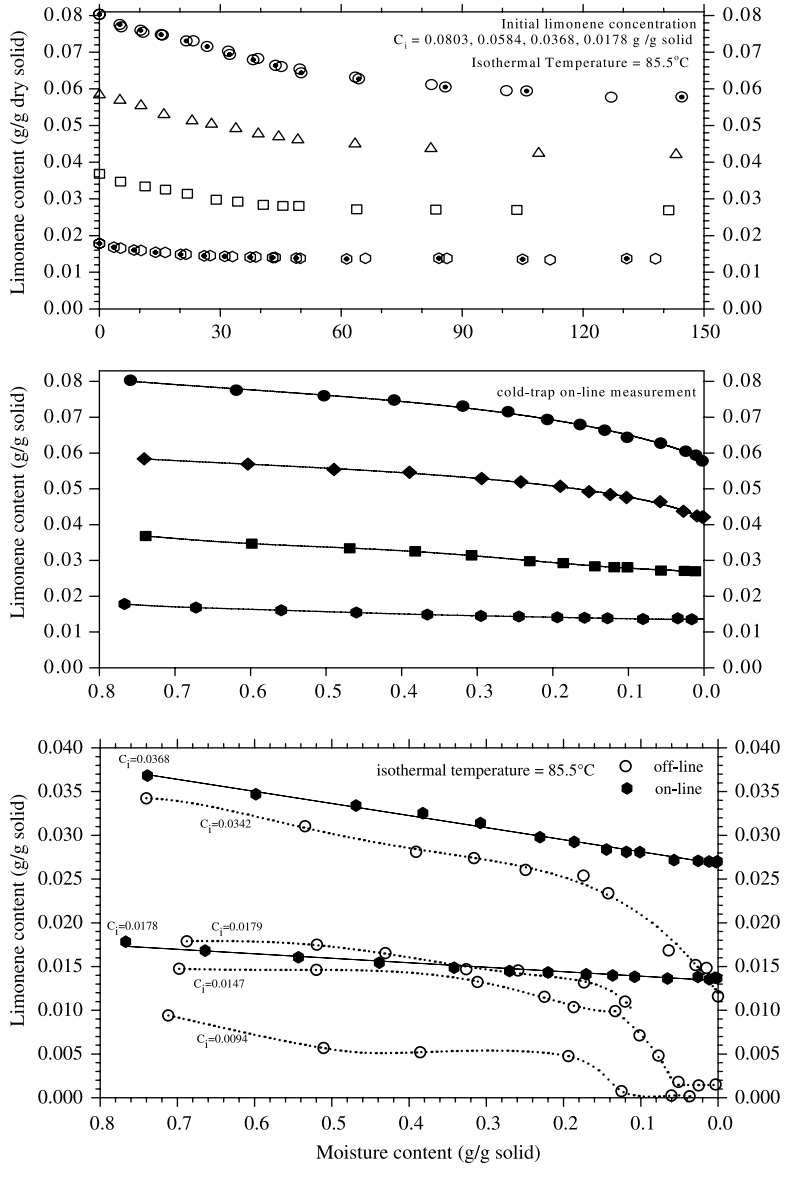

Figure 18.7 *Limonene concentration in formulated dough (porosity = 0.43 − 0.51) as a function of moisture content at 85.5°C as determined by off-line and on-line measurement. (Fu and others 2003b).*

concentration C_0:

$$C_\infty = 0.6563 \cdot C_0.002334 \text{ (g/g dry solid)}. \tag{18.13}$$

Note that when $C_0 = 0.0068$ (g/g dry solid), $C_\infty = C_0$. If the initial limonene concentration is greater than 0.0068 g/g solid, this predicts that 35% of the limonene can migrate out of the matrix (Fu and others 2003b). If the initial limonene concentration is less than or equal to 0.0068 g/g solid, no limonene should migrate at all. This amount

of limonene may be treated as bound limonene. It may interact with hydrophobic protein sites in the flour dough by a hydrophobic interaction.

Experimental data, which shows first-order behavior, can be mathematically modeled by either the rate constant or *D*-value (time required to reduce the concentration by 90%). Because the equilibrium concentration of limonene is a strong function of its initial concentration, comparing the rate of migration of limonene is more properly presented as a function of concentration driving potential, $C_0 - C_\infty$. As the *D*-value model ($r^2 = 0.965$) describes the data better than the rate constant model ($r^2 = 0.897$), further modeling will be done using *D*-values. Equation (18.11) relates the *D*-value to concentration driving potential, $C_0 - C_\infty$. Based on Equation (18.9) limonene concentration C_t can be calculated by Equations (18.10), (18.13), and (18.14), which results in Equations (18.15) and (18.16) after appropriate substitution:

$$D = 4712.7 \cdot (C_0 - C_\infty) + 12.98 \ (r^2 = 0.965), \tag{18.14}$$

$$C_t = 10^{-\frac{t}{D}} \cdot (C_0 - C_\infty) + C_\infty, \tag{18.15}$$

$$C_t = 10^{-\frac{t}{1619.76 \cdot C_0 + 1.98}} \cdot (0.3437 \cdot C_0 - 0.002334) + (0.6563 \cdot C_0 + 0.002334). \tag{18.16}$$

Predicted limonene concentration as a function of time at 85.5°C isothermal heating was calculated using Equation (18.16), and there is excellent agreement between experimental and calculated values (Fu and others 2003b).

The moisture concentration related to that *D*-value is also given and the *D*-value increases with a decrease in initial limonene concentration. The *D*-value in this paper is not diffusivity, but is the time for the concentration to change by 90%. Consequently, a decrease in *D*-value (less time) implies an increase in diffusivity that matches expectation. The *D*-values can be simply modeled by a second-order curve based on its trend. When the curve is extrapolated to zero *D*-value (*y*-axis), the initial limonene concentration is equal to 0.0064 g/g solid. This means that no flavor migration will occur when the initial limonene concentration is less than or equal to 0.0064 g/g solid (Fu and others 2003b). It is interesting to note that this value of 0.0064 g/g solid determined by extrapolating the curve to *D* equal to zero is very close to the previously determined value of 0.0068 g/g solid (determined by Equation (18.13)) at which limonene migration does not occur even with abundant water. Consequently, if the moisture content of the solid matrix remains greater than 0.1 g water/g solid, we would expect that all of the limonene would migrate out of the matrix. The set of experiments that illustrate this hypothesis is shown in Figure 18.8. Equation (18.17) also shows the results of a mathematical model based on the above assumption:

$$C_t = 10^{-\frac{t}{452}} \cdot (C_0 - 0.0068) + 0.0068 \ (D = 452\,\text{min}, C_\infty = 0.0068\,\text{g/g dry solid}). \tag{18.17}$$

There is excellent agreement between the model and experimental data from all four sets of experiments with moisture content greater than 0.10 g/g solid (Fu and others 2003b).

To validate the hypothesis that limonene is irreversibly bound in the dough matrix when moisture content is lower than 0.1 g water/g solid, an experiment was conducted

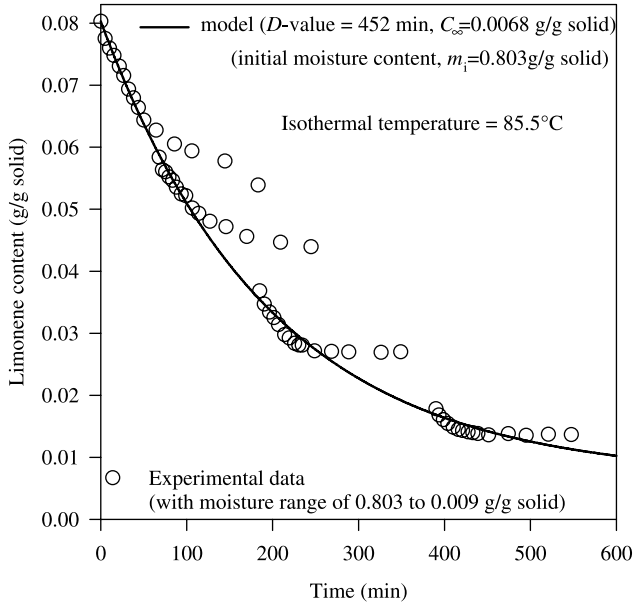

Figure 18.8 *Experimental and predicted limonene content as a function of time and D-value for formulated dough during isothermal heating at 85.5° C. (Fu and others 2003b).*

in which an unflavored dough was dried to 0.1 g water/g solid, and 0.0491 g limonene/g solid was added. The limonene was initially diluted with methylene chloride and then added to the dried matrix. The matrix was held at room temperature to evaporate the methylene chloride. The final weight difference was assumed to be the amount of added limonene. After the on-line experiments, the limonene was extracted from the matrix and its initial concentration was reconfirmed. The matrix temperature was brought to 85.5°C for about 90 min. The experiment shows that limonene can be irreversibly bound or physically entrapped in the dried matrix (Fu and others 2003b). Furthermore, the hydrophobic limonene is removed even more rapidly when water content is low. One further experiment was conducted to verify the hypothesis. The limonene-formulated dough was dehydrated to exactly 0.1 g/g solid and then rehydrated to 0.8 g water/g solid. The rehydrated sample was held for 4 h and then isothermally heated at 85.5°C for about 55 min. The result shows that flavor migration is precisely the same with or without added water (Fu and others 2003b). Thus, once the limonene has been entrapped, rehydration does not return the structure to its original configuration. This has been shown repeatedly for dehydrated solid-form foods (Van Arsdel and others 1973).

18.4.2 Temperature Dependence of Flavor Migration in Formulated Dough

There are two principal methods of describing the dependence of reaction rate constant or *D*-value on temperature:

1. The Arrhenius equation and
2. *z*-Value (°C temperature change to change the rate by a factor of 10) (Lund 1975).

Temperature dependence of limonene migration was determined for the highest concentration (0.0803 g/g solid) samples only. To fit these data to a linear model, equilibrium concentration was a function of temperature. Although there is no theoretical basis for this relationship, it is interesting to observe that C_∞ for limonene decreased linearly with decreased isothermal heating temperature. The lower the isothermal temperature, the smaller the equilibrium content and the greater the concentration driving potential, $C_0 - C_\infty$. The following equation relates C_∞ to isothermal heating temperatures:

$$C_\infty = 0.0005665\left(\frac{\text{g/g solid}}{^\circ\text{C}}\right) \cdot \text{temperature } (^\circ\text{C}) + 0.0006149 \text{ g/g solid.} \qquad (18.18)$$

An excellent agreement exists between predicted limonene and pyrazine concentration and experimental data (Fu and others 2003c).

For temperature dependence, the activation energies of limonene (58.2 kJ/mol) and pyrazine (51.5 kJ/mol) are greater than that of water (46.4 kJ/mol) at 85.5°C. For the *D*-value, the *z*-values of limonene and pyrazine are 39.5°C and 44.4°C, respectively. Given the same final moisture content of dried sample, more flavor would migrate from the matrix with lower isothermal heating temperature and longer heating time. This may be due to differences in structural changes caused by combinations of isothermal heating temperature and heating time. However, structural differences among samples dried under different isothermal heating temperatures were not apparent to the naked eye.

The area of aroma retention during drying processes, in particular for spray-drying and freeze-drying of food liquids, has been studied extensively by Karel, Thijssen, King, and others over several decades (Bruin 1992). Selective diffusion (Thijssen and Rulkens 1968; Thijssen 1970, 1971) and microregions (Flink and Karel 1970a,b) are two basic mechanisms that have been proposed to explain retention of homogeneously dissolved volatile aroma components during drying of liquid food materials. Most research has focused on liquid solutions and food gels such as extracts, juices, and milk. However, the retention of aroma in structured foods has received little attention. Neither of these two mechanisms explained the results of our experiments. Structural changes in dried samples in our case is likely different than those in spray-drying and freeze-drying. Another possible phenomenon that could influence flavor release or retention is a phase transition for the water–polymer matrix. The glass transition temperature (T_g) characterizes a transition from a solid "glassy" state to a liquid-like "rubbery" state when heated to temperatures greater than T_g. State diagrams show relationships between product composition and its physical state. At a temperature above T_g, the main consequence is an exponential increase in molecular mobility and free volume, and a decrease in viscosity in the rubbery state that affects various physico-chemical properties (Roos and Karel 1991a,b,c; Slade and Levine 1991). Increased molecular mobility also improves diffusion. When the temperature of a polymeric melt drops suddenly to a point below its T_g, the polymer backbones are immobilized. Molecules of amorphous food components are "entrapped" in the high-viscosity solid glass (Slade and Levine 1991; Roos 1995). Many state diagrams for starch and protein are available. For example, a state diagram of pregelatinized wheat starch has been constructed by Zeleznak and Hoseney (1987). As isothermal heating temperature is decreased, it is predicted that the moisture content for that transition state would be greater than for higher isothermal heating temperature. From the state diagram, the lower the isothermal heating temperature, the greater the moisture content corresponding to the glassy state. Thus, if the transformation from rubbery to glassy

states is related to flavor encapsulation, then C_∞ would increase as temperature of isothermal heating is decreased. However, in our case, lower isothermal temperatures result in lower C_∞ and thus a higher concentration driving potential, $C_0 - C_\infty$. Further experiments were not conducted to explain these observations.

The flour dough matrix (50% flour and 9% starch) is more hydrophilic than limonene. Based on formulation, water content ($\sim$0.72 g/g solid) is about 10 to 40 times higher than that of limonene (0.018–0.08 g/g solid) and 30 to 36 times higher than that of pyrazine (0.024– 0.0199 g/g solid). Based on mixing procedures, the flavor compound was the last ingredient added into the mixing bowl before mixing. After starch gelatinization, the water hydrates the hydrophilic sites of the nonaqueous constituents (flour matrix) by water–dipole associations. Similarly, limonene interacts with hydrophobic sites (protein) in the flour-dough matrix by a hydrophobic interaction. As discussed in a previous paper, 0.0068 g limonene/g solid was determined to be bound limonene (Fu and others 2003b). On the other hand, pyrazine is a hydrophilic compound and interacts with water and polar sites. Figure 18.9 (upper graph) shows the comparison of the *D*-values of limonene, pyrazine, and water as a function of initial concentration C_0, and concentration driving potential $C_0 - C_\infty$. When *D*-value is presented as a function of initial

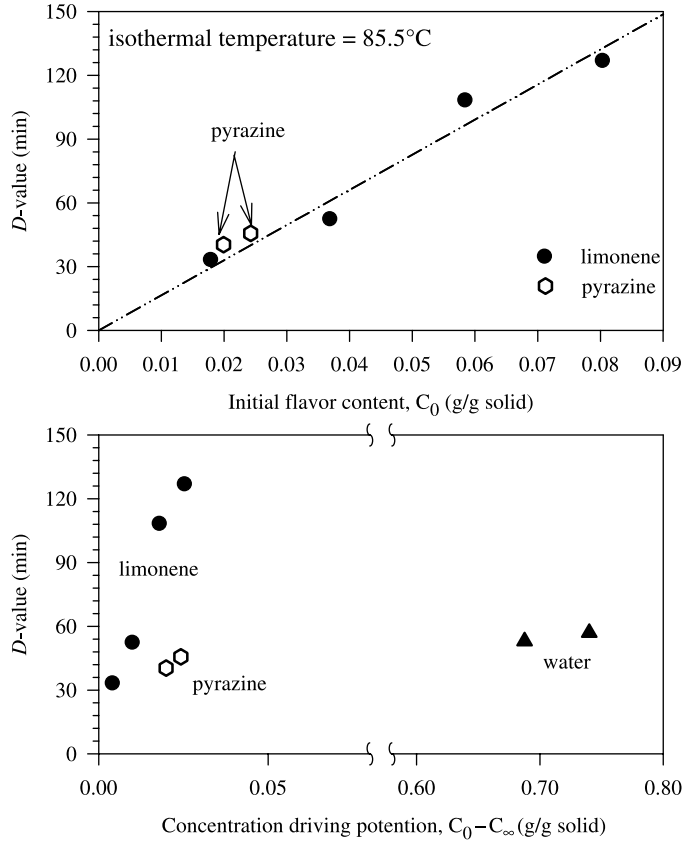

Figure 18.9 *D-value of water, pyrazine and limonene as a function of initial flavor content and concentration driving potential at 85.5°C isothermal temperature. (Fu and others 2003c).*

concentration, it appears that the migration rate of pyrazine is similar to that of limonene because the equilibrium concentration of limonene is a very strong function of its initial concentration. Consequently, to compare the rate of migration among these three components, it may more properly be presented as a function of concentration driving potential, $C_0 - C_\infty$. From the lower graph in Figure 18.9 it can be seen that the migration rates of pyrazine, limonene, and water are very different. The fourth point for the three components is the origin (i.e., the *D*-value is equal to zero when there is no concentration driving potential, $C_0 - C_\infty = 0$). The hypothesized bound limonene, 0.0068 g/g solid, was not an appropriate choice as the equilibrium concentration for all initial concentrations because the experiments that resulted in this value produced a matrix that was dried, and consequently the structure had changed. In these experiments, the migration rate decreased and leveled off at a much higher value. When the moisture content of the matrix was reduced to 0.1 g/g solid, the limonene was already entrapped. As shown in a previous paper (Fu and others 2003b), grinding did not bring about the release of entrapped materials. However, loss of volatiles does occur when the samples (with or without grinding) are heated or exposed to moisture (rehydration). The observation that the rate of flavor loss is much smaller than that before structural change indicates that the ability to release encapsulated volatile is dependent on the integrity of the dried structure. A dried limonene–dough matrix, when rehydrated, is unable to regain its original configuration, and, consequently, additional flavor will not be released even during reheating. For porous bodies, the Knudsen diffusion model can be used when the size of the pores is the order of the mean free path of the diffusing molecule. A relationship that describes Knudsen diffusion and its diffusion coefficient has been developed (Welty and others 1984), and the relation between diffusion coefficient and molecular weight is given by

$$D_{k,\text{eff}} \propto \sqrt{\frac{1}{M}}. \tag{18.19}$$

Therefore, based on molecular weight, the greater the molecular weight of a compound, the lower its diffusivity and the lower its migration. Thus, for molecular diffusion, the migration rates of limonene (MW = 136) and *tert*-butylbenzene (MW = 134) are smaller than that of water (MW = 18). In trying to obtain an accurate picture of the behavior of flavor chemicals in a food system, the volatility of these substances must also be considered. Water is the most important solvent in food products. The volatility of insoluble components is purely a function of the vapor pressure (or the boiling point as an approximation). The boiling point of limonene (bp = 176°C) is higher than that of water (bp = 100°C), so the migration rate of limonene should be smaller than that of water, based not only on the effect of molecular weight but also on the effect of volatility. For soluble components, such as pyrazine, volatility is a function of the partial vapor pressure and hence the concentration of the component in the solvent. The boiling points of limonene (bp = 176°C) and *tert*-butylbenzene (bp = 169°C) are very close and both are greater than that of pyrazine (bp = 115°C). However, volatility of pyrazine and limonene based on their boiling point cannot be compared because pyrazine is soluble and the solubility of limonene is negligible compared to that of pyrazine. The evaporation of soluble components, such as pyrazine, represents an ordinary distillation process, where volatility is a function of the partial vapor pressure and hence the concentration of the component in the solvent. Consequently, there is no single effect that explains the rate of migration of water, pyrazine, and limonene.

18.5 MICROWAVE HEATING OF FLAVOR-FORMULATED DOUGH

With conventional cooking methods, we have high-temperature ambient air (e.g., 180°C) at a rather low relative humidity. Heat permeates the surface and there is a temperature gradient and a corresponding water vapor pressure gradient directed towards the center. Because water vapor density is highest near the surface, this results in a pressure gradient that creates a driving force from the surface toward the center (Wei and others 1985a,b), thus helping to retain volatiles within the product. Due to the high ambient temperature, surface dehydration, protein denaturation, starch gelatinization, caramelization, and so on take place, and result in the formation of a crust (Van Eijk 1992). When collapse of the surface occurs, a sealing surface layer surrounds the food product and prevents or delays further evaporation of the water vapor and the associated flavoring substances into the ambient air. The very subtle but mouth-watering flavor nuances found in oven-baked products are largely due to flavors generated from the Maillard reaction. The Maillard reaction, which encompasses a complex series of reactions that starts with the condensation of amino acids and reducing sugars, has long been used as a tool for reproducing, enhancing, and improving mother nature's handiwork in a whole variety of food products. For the nonenzymatic browning or Maillard reaction to occur, the moisture content of the food product's surface must be greatly reduced (water activity levels between 0.6 and 0.8), and the surrounding air cannot be saturated with moisture (Risch 1989). There is no distinct temperature that must be attained for browning to occur; however, the higher the temperature, the greater the extent of browning.

18.5.1 Lack of Crispness (Texture) and Browning (Color, Flavor) of Microwave Foods

It is indisputable that microwave heating has many advantages over conventional heating, but the process itself is extremely complicated (Fu 2004). The well-recognized shortcomings of a microwaveable product compared to a conventionally prepared product are lack of surface browning and crisping, flavor loss, and flavor distortion. Microwave toughening is most probably related to moisture migration and loss in these reheated baked products, which can also lead to other undesirable protein–protein interactions. The lack of conventionally styled browning and crisping in microwave ovens is due to the microwave frequency used. At 2450 MHz, the wavelength, 12.2 cm, is too long to create the intense surface heat that occurs at the higher infrared frequencies, limiting the food item to a temperature of approximately 100°C. This is ideal for wet foods like vegetables and stews, but unacceptable for pastry, breaded or batter-coated items, and roast meat. In contrast to convectively heated food, we have relatively low-temperature ambient air (60–75°C) with a rather high relative humidity in most cases during microwave heating. The level of maximum temperature and consequently of maximum water vapor pressure generally lies further below the surface. The main driving force, therefore, is directed towards the surface instead of towards the center (Wei and others 1985a,b). Water vapor generated inside the food continuously migrates to the surface, drawing flavoring substances with it on the way out. The evaporation rate of water is not high enough to dry out the surface, and the evaporated water is continuously replaced by migration of water from the inside (Van Eijk 1992). For foods that require a long heating time, such as meat joints, the effect can be significant, and the resulting moisture loss from the surface of the product can be appreciable. An electromagnetic phenomenon creating "hot" and

"cold" spots is inherent in all microwave ovens and is responsible for much of the uneven cooking associated with them. Liquid products quickly dissipate the microwave energy and result in a more uniform product. Solid food products, multiphase systems, or frozen products develop hot and cold spots during heating, which further complicates flavor delivery in these systems (Steinke and others 1989).

During microwave heating the low surface temperature, its much higher water activity (approximately 1.0), and the lack of prolonged baking time have the following consequences:

1. No crust is formed because the necessary physical changes (protein denaturation, starch gelatinization, and so on) are inhibited, and
2. The formation of many flavor compounds and/or pigments (Maillard browning reactions) does not occur to the required extent.

Thus, some flavors that typically develop in a conventionally cooked product will not necessarily work in a microwaved product. Van Eijk (1992) states that the differences in flavor generation and the performance of flavoring substances in microwave foods can be explained satisfactorily by the differences in heating pattern, the corresponding differences in water vapor migration, and the resulting physical changes, particularly at the surface of the food. No athermal effects have been observed. To date, some very sophisticated packaging along with advanced susceptor technology has been the predominant solution to the lack of conventionally styled browning and crisping. Susceptors rapidly heat to temperatures where browning readily occurs and thus help produce flavor in the product. However, susceptors solve the flavor-related problems only on the surface.

Linking the formation of roast or baked flavor notes only to Maillard reactions is an oversimplification. The reactions of fats with other food constituents, for example, in meat, are also of great importance for the ultimate flavor profile. Because reactions of this type are also lacking in microwave cooking, an incomplete flavor profile may result. The ability to simulate a specific flavor in a food is significantly influenced by the flavor-binding capacity of the protein used. Denaturing of the protein can enhance flavor absorption. This probably reflects the greater exposure of hydrophobic segments of the protein because hydrophobic interaction is the principal force in the random coil-folding of proteins and accounts for binding of nonpolar flavor compounds. The extent that different proteins bind flavors cannot always be predicted in complicated food systems, because the presence of other factors (salts, lipids) will influence flavor behavior. Indeed, the moisture content of the system can influence the extent of aroma released.

18.5.2 Evaporative Cooling and Steam Distillation

During the heating process of foods containing water, the resulting evaporation at the surface causes a depression of the temperature, known as evaporative cooling. The surface of food is seen to be cooler than the region just below the surface and warmer than the surrounding air. This phenomenon is readily seen during the cooking of a meat roast (Nykvist and Decareau 1976; Nykvist 1977). At the same time, this surface evaporation can cause steam distillation of certain flavor components. Flavor release in microwave cooking is increased by steam distillation. In microwave heating, water vapor (steam) is one of the most important transport mechanisms contributing to the movement of flavor compounds within a food matrix (Fu and others 2003a,b,c). Individual compounds that make up a flavor that are particularly low in molecular weight and

water-soluble may be driven off or steam-distilled out of the product during microwave heating. Fruit and other "sweet" flavorings are more of a problem. They evaporate easily in foods with high initial water content because they contain a great number of short-chain, volatile flavoring substance. Moreover, they are often of a more hydrophilic character, and therefore a great part of the flavoring substance migrates to the aqueous phase of the food, which selectively absorbs the greater part of the microwave energy (Van Eijk 1992). The percentage loss may range from less than 10% for high-boiling compounds to 95% for very volatile compounds (Risch 1989). The latter are the ones that create a strong aroma, which is necessary when the flavor is designed to impart a balanced aroma profile in the room during microwave heating (Steinke and others 1989). In this case the flavor was added solely for aroma generation and contributed very little to the flavor profile of the microwave product itself. However, this phenomenon, flash-off, often leads to imbalance of flavor concentrations in a finished product with a different character from the flavor that was added before cooking. Formulations that compensate for flash-off may require a highly imbalanced flavor character prior to microwaving. The specific loss is dependent on the types of flavor components used and the food system in which it is incorporated. Moreover, the amount of flash-off can be highly variable within a product because temperature at any given moment can be quite local. As the outward migration of water vapor is the most important factor influencing flavor retention in the food product, the flavorings used for microwave application should have low water-vapor volatility unless the flavorings are intended to create the "oven aroma" of conventional cooking methods, or to cover undesirable off-notes released during microwave cooking.

18.5.3 A Case Study: Migration of Limonene in Formulated Dough Undergoing Microwave Reheating

Microwave reheating of ready-made cakes or desserts is one of the main domestic applications of microwave heating. The advantage is the ability of microwaves to penetrate a product and produce heat from within, thus generating heating profiles unattainable through conventional methods (Gerling 1986). The preparation time for microwave reheating is generally shorter than that of conventional heating. Loss of desired flavor may be associated with many flavor systems during microwave reheating. Fu and others (2003c) initiated an experiment by considering the migration of limonene during microwave reheating. Consequently, a series of experiments were performed to determine temperature and flavor-compound profiles in dough samples exposed to microwave energy. These experiments were limited in scope because

1. Reheating time was short (30 s to 2 min) and
2. Moisture content could not be less than 0.2 g water/g solid because the solvent extraction method did not totally remove the flavor compounds from the sample.

Three different reheating times and power combinations (335 s/600 W, 470 s/400 W, and 200 s/150 W) were used to create significantly different temperature profiles. Samples could then be created by cutting three shells with local moisture content greater than 0.2 g/g solid. Temperature profiles of three microwave reheating samples are shown in Figure 18.10. Due to microwave energy focusing on the central axis, the geometric

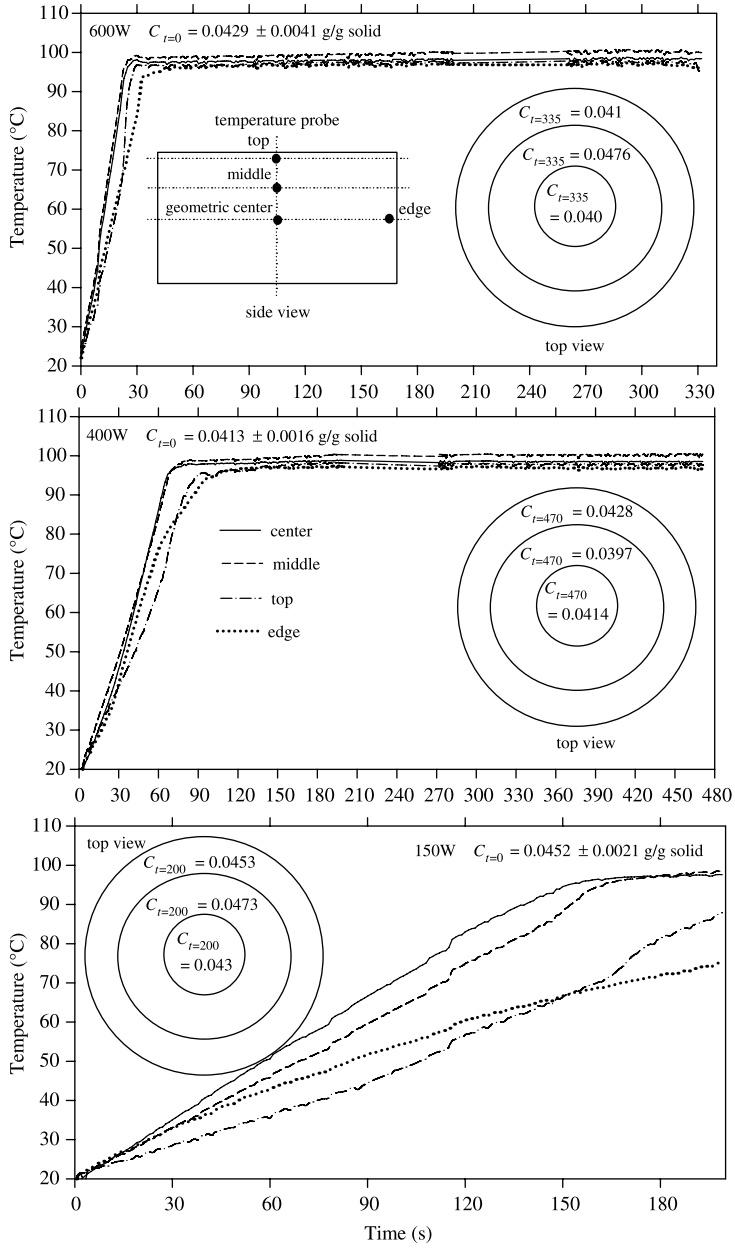

Figure 18.10 *Temperature profile of microwave heated limonene dough at 600, 400, 150 W. (Fu and others 2003c).*

center heated faster than the top of the center axis and edge. The temperature of the middle shell was between that of the center and outer shells. With higher power, 400–600 W, a 10–20°C difference between the highest and lowest temperatures was generated but lasted less than 20 s. As expected, eventually, the sample temperatures at all locations attained 95–100°C. With very low power (200 s/150 W), a 25°C temperature difference was

created for less than 70 s and the sample was at 95–98°C for less than 30 s. As expected, this sample exhibited greater temperature-difference profiles than those exposed to higher power levels. Regardless of different heating times and power combinations, the results show no significant limonene concentration difference between these three shells and also less than 1% overall change in total limonene content as a result of microwave reheating. Consequently, from a practical standpoint, there is essentially no loss of limonene from microwave-reheated dough-matrix samples.

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Section V

Beverages

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

19

Carbonated Beverages

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19.1 Background Information 420
19.1.1 History of Soft Drinks 420
19.1.2 Soft Drink Facts and Figures 421
19.1.3 Carbonation Science 421
19.1.4 Process Overview 422
19.2 Raw Materials Preparation 423
19.2.1 Concentrate 423
19.2.1.1 Simple Mixtures 424
19.2.1.2 Extracts 424
19.2.1.3 Emulsions 424
19.2.2 Water 425
19.2.2.1 Conventional Lime Treatment Systems (CLTS) 427
19.2.2.2 Membrane Technology 427
19.2.2.3 Ion-Exchange 428
19.2.3 Sweeteners 429
19.2.4 Carbon Dioxide 432
19.3 Syrup Preparation 434
19.3.1 Using Granulated Sucrose 434
19.3.2 Using Liquid Sugars 435
19.3.3 Medium Invert Sugar 435
19.3.4 Using High-Fructose Syrups 436
19.4 Carbonation 436
19.5 Filling, Sealing, and Packing 437
19.6 Quality Control and Assurance 439
19.7 Finished Product 440
References 441

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19.1 BACKGROUND INFORMATION

19.1.1 History of Soft Drinks

The first carbonated beverage, of sorts, was provided by nature, and dates back to antiquity, when the first carbonated natural mineral waters were discovered. However, they were not usually used for drinking. Instead, they were used for bathing by the ancient Greeks and Romans, owing to their purported therapeutic properties. It was not until thousands of years later, in 1767, that the British chemist, Joseph Priestley, was credited with noticing that the carbon dioxide he introduced into water gave a "pleasant and acidulated taste to the water in which it was dissolved" (Jacobs 1951). The history of carbonated soft drinks (CSDs) is somewhat sparse during its early evolution, but most agree that development of CSDs is due, in large part, to pharmacists.

Today, carbonated beverages are primarily recognized for their refreshing and thirst-quenching properties. In the early to middle 1800s, however, it was these pharmacists that experimented with adding "gas carbonium", or carbon dioxide, to water, and supplementing its palatability with everything from birch bark to dandelions in the hopes of enhancing the curative properties of these carbonated beverages (NSDA 2003). The term "Soft drinks", a more colloquial yet very common name for carbonated beverages, distinguishes them from "hard drinks", as they do not contain alcohol in their ingredient listing (NSDA 1999). This is in clear contrast to other beverages, such as distilled spirits, beer, or wine. These nonalcoholic, carbonated beverages are also called "pop" in some areas of the world, due to the characteristic noise made when the gaseous pressure within the bottle is released upon opening of the package (Riley 1972). Figure 19.1 provides a brief illustration of the major milestones in the history of American soft drinks. CSDs, pop, soda . . . whatever the moniker given to these beverages, one thing is clear: they have been an important part of our popular culture for decades, and will continue to be for many years to come.

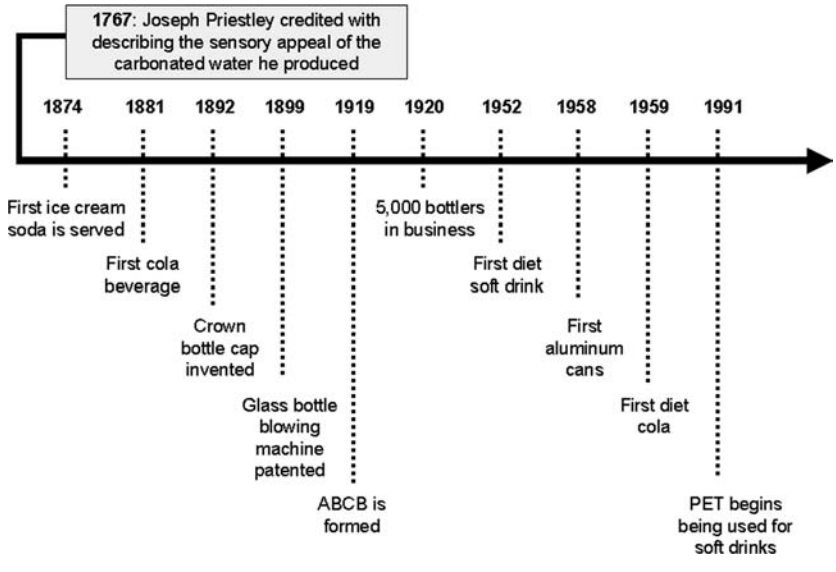

Figure 19.1 *Key milestones in the U.S. beverage industry.*

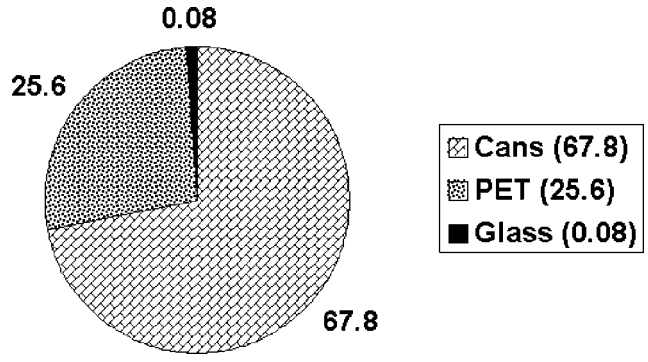

Figure 19.2 *Distribution of cans, PET, and glass CSD packages.*

19.1.2 Soft Drink Facts and Figures

Few people consciously consider how something as ostensibly simple as "soda pop" can markedly affect the economy on several fronts. The National Soft Drink Association (NSDA), founded in 1919 as the American Bottlers of Carbonated Beverages (ABCB), today represents hundreds of beverage manufacturers, distributors, franchise companies, and support industries in the United States. According to NSDA, Americans consumed nearly 53 gallons of carbonated soft drinks per person in 2002, and this translated into retail sales in excess of $61 billion. Nearly 500 bottlers operate across the United States, and provide more than 450 different soft drink varieties, at a production speed of up to 2000 cans per minute on each operating line! Figure 19.2 summarizes the apportionment of total soft drink production in the year 2000.

Finally, as an industry, soft drink companies employ more than 183,000 people nationwide, pay more than $18 billion in state and local taxes annually, and contribute more than $230 million to charities each year. Few could argue that the soft drink industry has not earned its place in the history of the American (and global) economy!

19.1.3 Carbonation Science

Before discussing the process of manufacturing carbonated soft drinks, it is important to establish some fundamental chemical/physical concepts with regard to the carbonation process itself. Simply put, in the beverage industry, "carbonation" is the introduction of carbon dioxide gas into water, as depicted in Figure 19.3.

The favorable results of this simple combination are many:

1. The carbonation provides the characteristic "refreshing" quality for which carbonated beverages are most popular;
2. The dissolved carbon dioxide acts as both a bacteristat and a bactericide, and

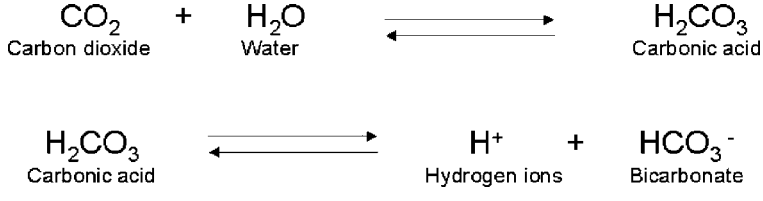

Figure 19.3 *Carbonation reactions.*

3. The carbon dioxide dissociates in aqueous media to form carbonic acid, which depresses the pH of the solution, thereby making the product even more protected from microbial harm (Granata 1946).

All in all, from a microbiologic perspective, carbonated soft drinks are innately very safe beverages. Once the carbon dioxide is introduced into the water, which will ultimately join with flavors and sweeteners to form the complete beverage, the beverage technologist must understand how to measure and express the level of carbonation. The accepted convention in the beverage industry is *not* to measure carbon dioxide as a true concentration, expressed in parts per million, or milligrams per liter. Instead, carbonation is expressed in units called "volumes". The concept is ultimately based on the physical gas laws of Henry, Boyle, and Charles, wherein pressure, temperature, and volume are closely interdependent. The colder the liquid, the more gas can be dissolved within it. Even within the industry, however, there is some confusion over what the exact definition of a "volume" is (Medina 1993), usually arising from the temperature included in the definition. For our purposes, we will define one "volume" based on the Bunsen coefficient, described by Loomis as, "the volume of gas (reduced to 0°C and 760 mm) which, at the temperature of the experiment, is dissolved in one volume of the solvent when the partial pressure of the gas is 760 mm" (Loomis 1928). More informally, and to put this concept in perspective, consider a 10-oz bottle of carbonated beverage, representing roughly 300 mL of liquid. If this carbonated beverage is prepared at one "gas volume", the package would contain approximately 300 cc of carbon dioxide. We would consider this very low carbonation from a sensory perspective, and would have a barely noticeable "fizz" upon removal of the closure. Imagine, however, for the same 300 mL of liquid, we carbonate to four gas volumes (a level typical of many products on the market today). This means that roughly 1200 cc of carbon dioxide has been introduced into the same 300 mL volume of liquid. More gas, into the same amount of liquid, and the same vessel size – imagine the increase in pressure contained within the bottle. This example explains why the characteristic "pop" of soda pop is heard when a bottle is uncapped, or a can is opened!

For the purposes of this text, the discussion of carbonation has been somewhat oversimplified, in order to make the concept more easily understood. As with any industry, the more one investigates any given topic, the more complicated and scientifically intense the subject usually becomes. Carbonation, for example, can be affected by a variety of factors, including other solids present in the liquid being carbonated, temperatures of the gas and the liquid, atmospheric pressure/altitude, and how far carbon dioxide varies from ideal gas behavior (Gidden 2001). These are cited merely for consideration, but are outside the scope of this manuscript.

19.1.4 Process Overview

The process of manufacturing carbonated beverages has remained fundamentally the same for the last several decades. Certainly, new equipment has allowed faster filling speeds, more accurate and consistent fill heights, more efficient gas transfer during carbonation, and other improvements, but the process remains one of cooling water, carbonating it, adding flavor and sweeteners, and packaging it in a sealed container. Figure 19.4 illustrates the overall process in somewhat more detail, as we continue to build upon the basic foundation we will be discussing throughout this chapter. As we proceed, the figures depicted will become more complete, as each critical process to carbonated beverage manufacture is explained.

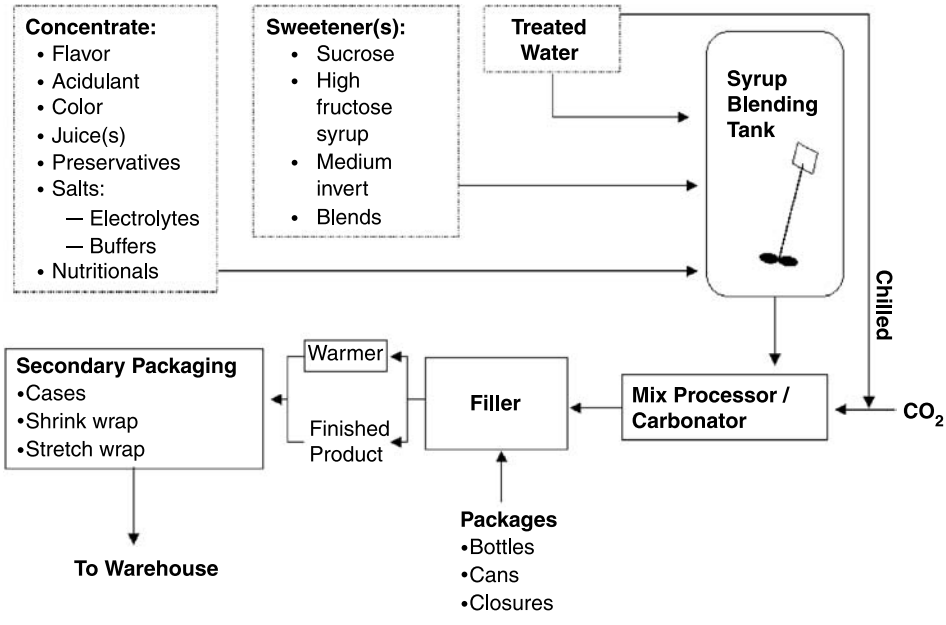

Figure 19.4 *Process overview of carbonated beverage manufacture.*

Carbonated beverage production begins with careful measurement of the formula quantities of each component into the syrup blending tank. Critical components include the *concentrate*, which contains the bulk of the flavor system, the *sweetener*, which typically includes the nutritive sweeteners high-fructose syrup or sucrose (in the case of diet beverages, these are replaced with one of the high-potency sweeteners available), and *water*, which generally begins as municipal drinking water, and is further purified within the beverage plant. These are then blended to assure homogeneity of the batch according to carefully prescribed standard operating procedures. Once blending in the syrup tank is complete, the "finished syrup" is tested for correct assembly, then pumped to the mix processor, where the syrup is diluted to finished beverage level with chilled, carbonated, treated water (often a 1 : 6 dilution of syrup:treated water, although this varies by product). After this, the now carbonated beverage-level solution proceeds to the filler, where it is fed (usually volumetrically, by gravity) into bottles or cans, then sealed (capped in the case of bottles, seamed in the case of cans). The finished product is then either passed through a warmer, in order to prevent excessive condensation from forming (depending on the type of secondary packaging used), or sent directly to secondary packaging. This can include plastic or cardboard cases, shrink wrap, stretch wrap, or even more innovative devices. After packaging, the product is palletized, and stored in the warehouse until it is ready for distribution.

19.2 RAW MATERIALS PREPARATION

19.2.1 Concentrate

In the carbonated soft drink industry, "concentrate" is the name given to a mixture of many different categories of ingredients, as illustrated in Figure 19.5. The most notable of these,

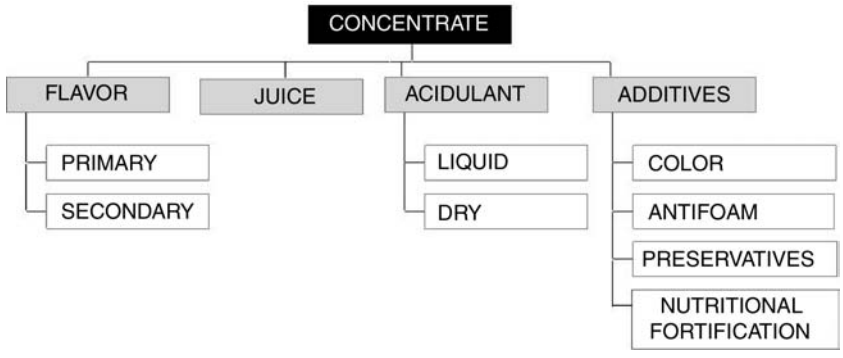

Figure 19.5 *Concentrate components.*

and, indeed, the topic of many urban legends surrounding its utter secrecy, is the flavor component. This is where the proprietary formulations of essential oils are found, which combine to form the characteristic flavor of the trademark beverage. Flavor components can include a single, "primary" component, or be distributed in various ways among multiple components, for example, a high-potency sweetener supplied as a dry salt as part of a secondary flavor component. In general, the majority of flavor systems include primary flavor components, and these fall into three broad categories:

1. Simple mixtures,
2. Extracts, or
3. Emulsions.

19.2.1.1 Simple Mixtures. These are perhaps the simplest of the flavor categories to understand, but they also represent the minority of those in existence. Here, a combination of miscible liquids, or easily soluble solids, are blended together to form a homogeneous aqueous mixture. Because so many essential flavor oils are not readily water soluble, the beverage technologist must abandon the idea of the simple mixture for one of the other, more flexible categories of flavors.

19.2.1.2 Extracts. As the name implies, this category of flavors involves extracting the desired flavor constituents from essential oils. Simply put, the extraction solvent – usually ethanol (although sometimes propylene glycol is used) – is used to partition those flavor constituents that are soluble in the solvent, but not freely soluble in the water directly. In this way, these flavor compounds become fully dissolved in the ethanol first. Then, this ethanolic "extract" (which is, in effect, an ethanolic solution of the flavor compounds) is added to water. Because ethanol is freely miscible with water, it acts as a carrier vehicle to help dissolve or disperse the otherwise water-insoluble flavor constituents (Woodruff 1974). Today, equipment for both batch and continuous liquid extraction of flavor oils is available, and more novel approaches have also been developed (for example, gas extraction, supercritical fluid extraction, and other patented processes).

19.2.1.3 Emulsions. This third category is likely the largest, encompassing the bulk of the flavor systems available today. In the carbonated beverage industry, oil-in-water (or o/w) emulsions are the standard. This model involves an oil (lipophilic) internal phase, and an aqueous (hydrophilic) external phase, being made "compatible" by the use of a surfactant, or emulsifier. Surfactants are compounds that are amphiphilic; that is, there are

both hydrophilic and lipophilic portions on the same molecule! This facilitates a decrease in the surface tension when oil and water are mixed together, and also allows the lipophilic portion to align with the oil, and the hydrophilic portion to align with the water (Banker 1996). In so doing, the emulsifier forms a bridge, of sorts, between the two phases, and allows them to be dispersed, without gross separation, for the desired length of time (generally at least as long as the technical shelf-life of the beverage).

As carbonated beverages are of low pH, owing in part to the carbonic acid from the dissolved carbon dioxide, but also from the acid components of the formulas, acid hydrolysis is one of the major concerns to the beverage flavor developer. By positioning itself between the oil and water phases, the emulsifier protects the sensitive flavor oils from chemical degradation in this acidic environment. In addition, the emulsifier protects the flavor oils from oxidation from the naturally dissolved oxygen in the water that constitutes the aqueous phase. So, a well-designed and prepared emulsion can dramatically extend the sensory shelf-life of the flavor system, and the overall physical stability of the beverage.

In addition to the flavors, Figure 19.5 also depicts a variety of other components that may be part of the concentrate. These include juices, which must be handled and stored carefully in order to preserve their quality, acidulants (both liquid and dry), and a host of other additives, depending on their desired function (for example, antifoam, preservative, nutrients, and so on).

19.2.2 Water

Water is the major component in carbonated beverages, and represents anywhere from 85 to near 100% of the finished product. Interestingly, it is unlike any other ingredient, because we rarely have the number of options for water supply that we have with other raw materials! Obviously, then, particular diligence must be employed when selecting a water supply. Beverage plants use water from ground supplies, surface supplies, or both. Ground supplies include springs, deep and shallow wells, and artesian aquifers. Surface supplies include rivers, lakes, streams, and reservoirs. Within these sources, there is wide variation in type and content of inorganic (for example, metals, minerals, sulfate, chloride, nitrate), organic (for example, volatile organics, natural organic matter), microbiologic (bacteria, viruses, protozoa), and radiologics (radionuclides, alpha- and beta-activity) material. Table 19.1 provides a relative comparison of some characteristics of ground and surface supplies (Bena 2003).

One critical point of which to be aware is that municipal treatment plants should not normally be depended upon to consistently supply water suitable for the needs of most carbonated beverage manufacturers. Although the municipality treats the water so that it is safe to drink, and is esthetically pleasing to the consumer (potable and palatable), they cannot afford to consider the needs of all industrial end users, so they may not consistently supply a water of the high quality needed for producing our finished product and assuring the beverage a long shelf-life. There is also the possibility of contamination of the city water as it passes through the distribution system from the municipal treating plant to the beverage plant. This is particularly true with respect to organic matter and metal content, such as iron. The quality of the water used for carbonated soft drinks must be considered from several perspectives:

1. *Regulatory Compliance*. The water used must be in compliance with all presiding local and national laws and guidelines. The jurisdiction is generally clear in the United States, between the Environmental Protection Agency and the Food and Drug Administration. However, as you consider the international beverage locations, the regulatory picture sometimes becomes more cloudy.

TABLE 19.1 Comparison of Ground and Surface Water Supplies.

Parameter	Ground Water	Surface Water
Total dissolved solids	Higher	Lower
Suspended solids	Lower	Higher
Turbidity and color	Lower	Higher
Alkalinity	Higher	Lower
Total organic carbon	Lower	Higher
Microbiology		
Protection from bacteria and viruses	Highly protected	Highly susceptible
Protection from protozoa	Almost completely protected	Highly susceptible
Presence of iron and/or manganese bacteria	Common	Rare
Hydrogen sulfide gas	Common	Uncommon
Aeration/dissolved oxygen	Lower	Higher
Temperature	More consistent	More variable
Flow rate	Very slow (1 m/day)	Very fast (1 m/s)
Flow pattern	Laminar	Turbulent
Susceptibility to pollution through surface run-off	Low	High
Time for a contaminant plume to resolve	Very long, often decades, potentially centuries!	Usually short, days/months, sometimes years

Source: Bena (2003).

2. *Beverage Stability*. Intuitively, as the major ingredient in carbonated soft drinks, the constituents in water can have a profound impact on the overall quality and shelf-life of the beverage products. For example, if alkalinity is not controlled, the acidic profile of the beverage formulas will be compromised, making the beverage more susceptible to microbial growth and spoilage.
3. *Sensory*. Many contaminants, even at levels within drinking-water standards, may adversely affect the finished beverage. For example, some algae produce compounds (geosmin and methyl isoborneol) that are sensory active at levels as low as nanograms per liter (Suffet 1995). These can result in a "dirty, musty" flavor and aroma in finished products.
4. *Plant Operations*. Water for nonproduct (auxiliary) uses must also meet the performance standards of the carbonated soft drink producer. These standards and guidelines are usually enacted to prevent corrosion (for example, from high chloride content in heat exchangers) and scaling (for example, from hardness salts in boilers), which may result in premature equipment failure and/or loss of operational efficiency.

Whether the beverage plant has its own well, or the water supply comes from a modern municipal treatment plant, each individual water supply presents its own particular problems. In most, if not all cases, the incoming raw water that supplies a beverage plant already meets the applicable standards for potability of drinking water. The beverage producer then further purifies the water to meet the quality necessary for their products. This treatment can take many forms, but the three largest categories of in-plant beverage water treatments are

1. Conventional lime treatment systems (CLTS);
2. Membrane systems (including reverse osmosis, nanofiltration, and ultrafiltration); and
3. Ion-exchange.

Volumes have been written about each treatment modality, and a detailed discussion is beyond the focus of this chapter. However, a brief summary of each treatment category is provided in the following (Bena 2003).

19.2.2.1 Conventional Lime Treatment Systems (CLTS). This treatment chain represents the majority of most beverage treatment armadas worldwide, although the balance is quickly shifting in favor of membrane technologies. CLTS involves the addition of a coagulant (as an iron or aluminum salt), hydrated lime (for pH control), and chlorine (for oxidation and disinfection) to a reaction tank. The agitation is gently controlled over the course of a two-hour retention period, during which time a floc begins to form, grow, and settle, bringing contaminants with it to the bottom of the tank, where they await discharge. Figure 19.6 illustrates what happens in this reaction vessel.

Historically, and as little as 25 years ago, conventional lime treatment was regarded as the "ideal" treatment for raw water of virtually any quality. Indeed, this system, coupled with the required support technology – fine sand filtration, granular activated carbon, polishing filtration, and ultraviolet irradiation – does address a broad range of water contaminants. The advantages and disadvantages of conventional lime treatment are summarized in Table 19.2.

19.2.2.2 Membrane Technology. Clearly, this has seen the most growth in recent years with the advent of more resistant membrane materials of construction and more flexible rejection characteristics. Included in this category is the prototype of the cross-flow, polymeric membrane filtration systems – reverse osmosis, along with nanofiltration and ultrafiltration (both polymeric and ceramic). By carefully controlling the membrane pore size during manufacture, and applied pressure during operation, reverse osmosis

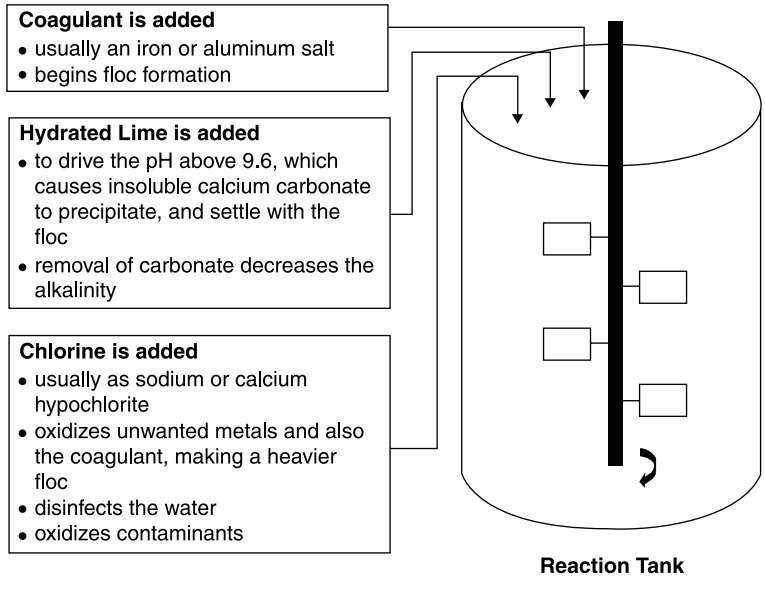

Figure 19.6 *Reaction tank in a conventional lime treatment system.*

TABLE 19.2 Advantages and Disadvantages of CLTS.

Advantages	Disadvantages
Removes alkalinity and hardness	Does not effectively reduce nitrate, sulfate, or chloride concentration
Removes organic debris, particulates, and natural organic matter (NOM)	Sludge formation and disposal requirements need to be addressed
Reduces metal concentrations (iron, manganese, arsenic, others) and some radionuclides	May promote the formation of disinfection byproducts (trihalomethanes) under certain conditions
Reduces some color compounds (tannins), off-tastes, and off-odors	Often difficult to operate consistently in waters with very low dissolved solids
Reduces bacteria, virus, and protozoan populations	These are relatively large space requirements on the plant floor ("footprint")

Source: Bena (2003).

membranes can effectively remove in excess of 99% of many dissolved species – down to the ionic level (for example, dissolved calcium or sulfate). Table 19.3 illustrates the relative capabilities of the three major membrane processes with regard to a variety of possible constituents in the incoming water (Brittan 1997).

Because reverse osmosis is often the cited "membrane standard" against which the performance of others are judged, the advantages and disadvantages of reverse osmosis are listed in Table 19.4. It is also worth mentioning, although not discussed here, that among this group are the "hybrid" technologies, which include novel membrane and ion-exchange utilization. Examples are electrodialysis technology for removal of ionic species in water, and continuous electrodeionization.

19.2.2.3 Ion-Exchange. This technology is routinely utilized for partial or complete demineralization of the water supply, softening, dealkalization, or it can be customized for selective removal of a specific contaminant (for example, denitratization). In simplest

TABLE 19.3 Relative Comparison of Reverse Osmosis, Nanofiltration, and Ultrafiltration.

Component	Reverse Osmosis[a]	Nanofiltration[a]	Ultrafiltration[a]
Alkalinity	95–98%	50–70%	None
TDS	95–98%	50–70%	None
Particulates	Nearly 100%	Nearly 100%	Nearly 100%
Organic matter	Most >100 MW	Most >200 MW	Some >2000 MW
THM precursors	90+%	90+%	30–60%
Sodium	90–99%	35–75%	None
Chloride	90–99%	35–60%	None
Hardness	90–99%	50–95+%	None
Sulfate	90–99%	70–95+%	None
Nitrate	90–95%	20–35%	None
Protozoa	Nearly 100%	Nearly 100%	Nearly 100%
Bacteria	Nearly 100%	Nearly 100%	Nearly 100%
Viruses	Nearly 100%	Nearly 100%	Nearly 100%
Operating pressure	200–450 psi	100–200 psi	80–150 psi

Source: Adapted from Brittan (1997).
[a]Approximate removal percentages; actual performance is system-specific.

TABLE 19.4 Advantages and Disadvantages of Reverse Osmosis.

Advantages	Disadvantages
Removes nearly all suspended material, and greater than 99% of dissolved salts in full-flow operation	Pretreatment must be carefully considered, and typically involves operating costs for chemicals (acid, antiscalant, chlorine removal)
Significantly reduces microbial load (viruses, bacteria, and protozoans)	Does not produce a commercially sterile water
Removes nearly all natural organic matter (NOM)	Membranes still represent a substantial portion of the capital cost, and may typically last 3–5 years
May be designed as a fully automated system with little maintenance	Low solids water may be aggressive toward piping and equipment, so this must be considered for downstream operations
It has relatively small space requirements on the plant floor ("footprint")	High-pressure inlet pump is required

Source: Bena (2003).

terms, ion-exchange involves using a selective resin to exchange a less desirable ion with a more desirable ion. Of course, a great deal of chemical research goes into the development of these selective resin materials, but the functional outcome remains straightforward. For example, softening resins are often employed to remove hardness (calcium and magnesium) from the water entering boilers and heat exchangers. In this application, the hardness ions are not wanted. The softening resin (for example, a sodium zeolite clay) is charged with active and replaceable sodium ions. When the "hard" water passes across the softening bed, the resin has a selectivity for calcium and magnesium, so it replaces them for sodium. The result is that the water exiting the softener is virtually free of calcium and magnesium (because they were replaced by sodium), and is safe to use in boilers and other equipment, as it will no longer have the tendency to form scale.

To supplement the major treatment systems mentioned above, the carbonated beverage producer often utilizes a host of other "support technologies", including activated carbon filtration (to remove organic contaminants and chlorine), sand filtration (to remove particulates), and primary and secondary disinfection (using chlorine, ozone, ultraviolet, heat, or a combination). By the time the treated water is finished, it is microbially and chemically safe, clear, colorless, and ready to be used for syrup and beverage production.

19.2.3 Sweeteners

The two major categories of sweetener are nutritive (that is, they provide some caloric value) and "high-potency" (that is, the type used in diet beverages, because they are many times sweeter than sucrose, and generally noncaloric). There are several high-potency sweeteners available to the worldwide beverage developer (aspartame, acesulfame potassium, and others), which are almost exclusively, if not always, included as part of the "concentrate" flavor system as a dry substance package. As such, their quality can be more easily controlled by the vendor, as with any of the other concentrate ingredients, and minimal intervention is needed at the carbonated soft drink manufacturing facility. These high-potency sweeteners, therefore, will not be addressed in this chapter. A concise treatise on the topic, however, is provided by the International Society of Beverage Technologists (Koch 2000).

Next to water, however, the nutritive sweeteners represent the second most prevalent ingredient in the finished beverage. The most common nutritive sweeteners used in the

carbonated soft drink industry are sucrose and high-fructose syrups, with sucrose (from cane or beet) being the most common internationally. Within the United States, nearly all of the nutritive sweetener used in carbonated beverages is high-fructose corn syrup (HFCS, either 42 or 55%). In 1996, the U.S. corn-refining industry produced over 21 billion pounds of high-fructose corn syrups, representing about 12% of the total corn crop (Hobbs 1997).

Although high-fructose syrups may be obtained from other starting materials, like wheat, or tapioca starch, corn remains the most prevalent starting material. A starch slurry is first digested by the addition of the α-amylase enzyme, which results in gelatinization and ultimate dextrinization of the starting starch. Then, glucoamylase enzyme is added, resulting in an enriched glucose syrup (95% glucose). After this the glucose syrup is purified via particle filtration, activated carbon adsorption, and both cation and anion exchange. Then, evaporation brings the solids content within range for effective passage through the isomerization column containing the glucose isomerase enzyme. This enzyme converts much of the 95% glucose syrup to fructose, which is again purified as before, and evaporated. The result is HFCS-55 of high quality. In some formulas and/or markets, HFCS-42 is used, which is simply a blend of the HFCS-55 with a 95% glucose stream to result in a product that is 42% fructose. The generic process by which corn starch is transformed to high-fructose corn syrup is illustrated in Figure 19.7 (Boyce 1986).

In general, HFCS-55 (55% fructose) is a highly pure ingredient, due, in large part, to the activated carbon, cation, and anion exchange steps required of the process. However, the most recent research highlights the occurrence of potent sensory-active compounds that could form via chemical or microbial pathways in HFCS, including isovaleraldehyde, 2-amino acetophenone, and maltol (Finnerty 2002). When properly produced and stored, no additional treatment is necessary at the beverage plant.

Sucrose, although the clear exception in the North American beverage industry, continues to be the mainstay for international beverage markets. It may be obtained from

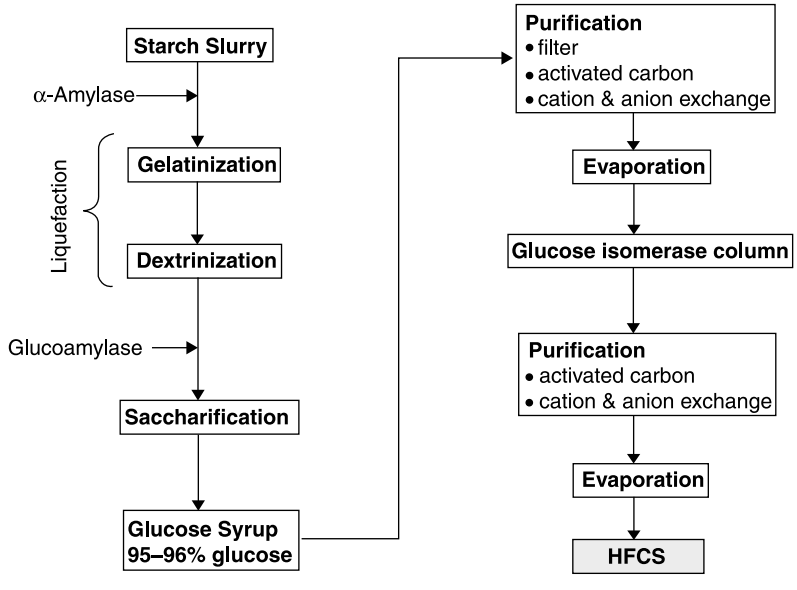

Figure 19.7 *High-fructose corn syrup manufacture.*

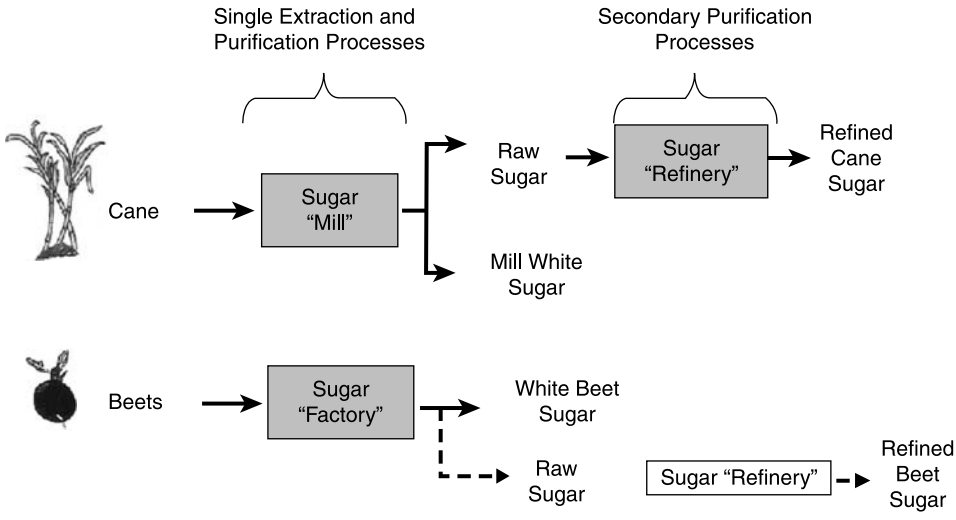

Figure 19.8 *Cane* vs. *beet sugar process flow.*

sugar cane or sugar beet, following two distinct separation and purification schemes, as depicted in Figure 19.8 (Galluzzo 2000). The three indicators of sucrose quality generally recognized by the sugar industry are color, ash, and turbidity. Internationally, depending on the quality of the available sucrose, it is not uncommon to subject the incoming granular or liquid sucrose to additional treatment at the beverage plant. Ash, or residual inorganic minerals, remains difficult to adequately treat at the carbonated soft drink plant, so great effort is made to source sucrose with an acceptable ash content (as defined by the individual company specifications). Turbidity is easily remedied at the beverage plant via an in-line filtration step, often incorporating diatomaceous earth as a filter aid. Color, considered by some as the primary indicator of sucrose quality, is also able to be treated at the beverage plant, but typically requires hot treatment through activated carbon. This removes color and many sensory-active compounds, and also serves to render the sucrose free of most viable microorganisms. Figure 19.9 (McLeod 2001) briefly summarizes the handling and treatment of sucrose at the carbonated soft drink facility.

Liquid sucrose, usually commercially available at 67 Brix concentration (67 Brix is equivalent to 67% sucrose, by weight), is sometimes used for the production of carbonated soft drinks. These are two distinct disadvantages of using liquid sucrose instead of granulated sucrose:

1. The end user ultimately pays for shipping 33% water, because the ingredient is only 67% sucrose solids, as compared to granulated sucrose, which is 100% sucrose solids, and
2. This water also means that the liquid has a higher water activity than granulated sucrose, making it much more susceptible to microbial spoilage. With liquid sucrose operations, absolutely diligent transport and handling procedures are imperative.

The last, less common type of nutritive sweetener used in this industry is medium invert sugar. Chemically, this product has similarities to both sucrose and high-fructose corn

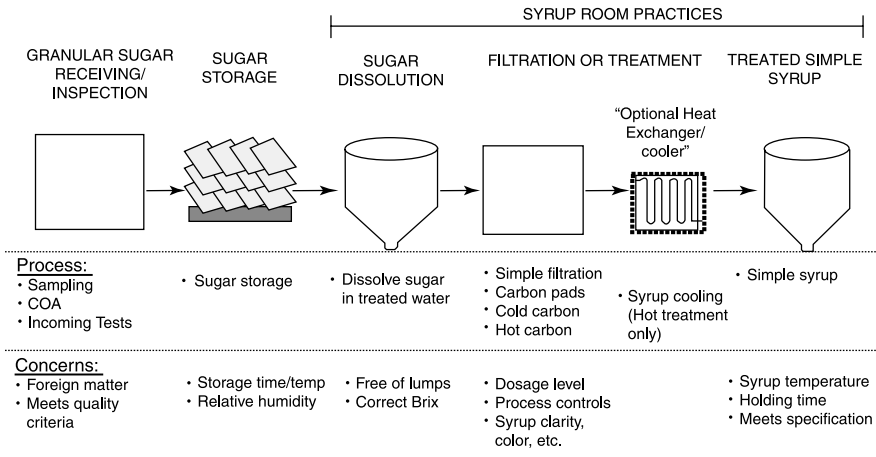

Figure 19.9 *Sucrose handling and treatment at the beverage plant.*

syrup. With medium invert sugar, or MIS, the starting material is liquid sucrose, which is then treated by one of three processes:

1. Heat and acid;
2. Ion-exchange; or
3. Invertase enzyme.

The end result of any of these processes is that roughly 50% of the starting sucrose is transformed into "invert sugar", an equimolar mixture of glucose and fructose. At this point, the inversion process is stopped, and the final commercial product contains 50% sucrose, 25% glucose, and 25% fructose. This gained favor over liquid sucrose in the beverage industry for two main reasons:

1. The finished material is 76 Brix, *vs.* 67 Brix for liquid sucrose, so less water is shipped; and
2. MIS has a much lower water activity, and is, therefore, much more microbiologically stable.

In summary, the producers of carbonated soft drinks have several options at their disposal for providing the sweetness to the consumer that is so characteristic of these products. Internationally, sucrose is the major sweetener used, but in the United States, high-fructose corn syrup is preferred. Irrespective of the type of sweetener, the beverage industry has treatment methods at its disposal to ensure that this ingredient consistently meets the high standards of chemical and microbial quality necessary to be used in the production of syrup and beverage.

19.2.4 Carbon Dioxide

At normal temperatures and pressures, carbon dioxide is a colorless gas, with a slightly pungent odor at high concentrations. When compressed and cooled to the proper temperature, the gas turns into a liquid. The liquid in turn can be converted into solid dry ice. The dry ice, on absorbing heat, returns to its natural gaseous state.

We learned a little of the history of carbonation earlier in this chapter, because the concept is so critical to the production of carbonated soft drinks. Just as critical is the quality of the carbon dioxide used in this application. For many years, the quality of carbon dioxide was minimized, largely because there were no uniformly available methods with which to test the gas, as an ingredient. Those procedures that were available required special expertise to properly sample and handle this cryogenic gas. Maintaining standards of quality of the carbon dioxide used in beverages was traditionally relegated to the U.S. Compressed Gas Association (CGA), whose "quality verification levels" were incorporated into the beverage company's specification system. Then, in 1999, the International Society of Beverage Technologists (ISBT) developed the Quality Guidelines and Analytical Procedure Bibliography for Bottler's Carbon Dioxide (McLeod 2001). This was a cross-corporate effort by carbon dioxide suppliers, end-users, testing labs, and allied businesses to completely update the obsolescent guidelines that had been recognized for decades. The guidelines are only available for purchase through ISBT (www.bevtech.org), but include parameters related to health/safety, sensory, and good manufacturing practices at the supplier.

Carbon dioxide may be obtained and purified from a number of different "feed gas" sources, the majority of which are listed in Table 19.5. There are other more "exotic" sources, which are often the result of carbon dioxide being generated as a side product during an organic chemical synthesis. In addition to commercial supplies, some carbonated beverage plants produce and purify their own carbon dioxide. The most common feed-gas sources for these applications are combustion (where the flue gas is recovered, concentrated, then purified) and breweries (where the CO_2 generated from microbial metabolism is recovered and purified). Whether supplied commercially or in-house, the carbon dioxide used in carbonated soft drinks is of high quality (greater than 99.9% CO_2); in most cases, it even exceeds that of medical-grade gas.

The liquid carbon dioxide that is delivered to beverage plants is generally stored in large "bulk receivers", which are vertically or horizontally oriented steel tanks with urethane foam or vacuum insulation. In the most common arrangement, carbon dioxide is withdrawn from the liquid phase at the bottom of the tank, and vaporized by one of several methods. Due to this withdrawal, the equilibrium between vapor and liquid in the tank remains dynamic. The air gases (oxygen, nitrogen) partition into the vapor phase of the vessel, and are routinely purged to maintain the purity of the carbon dioxide within the bulk receiver. Similarly, some components preferentially partition, in trace amounts, into the liquid phase of the carbon dioxide (liquid CO_2 is an excellent solvent). Many beverage plants choose to subject the freshly vaporized carbon dioxide to one final step of purification just prior to the point of use. This is usually a simple

TABLE 19.5 Feed Gas Sources for Carbon Dioxide.

Combustion
Wells/geothermal (natural CO_2 wells)
Fermentation (breweries, ethanol plants, and so on)
Hydrogen or ammonia plants
Phosphate rock
Coal gasification
Ethylene oxide production
Acid neutralization

Source: Adapted U.S. Compressed Gas Association (2000).

filtration through activated carbon alone, or through a mixed adsorbent bed of carbon (to remove organic contaminants); a silica-based desiccant (to remove moisture); and a molecular sieve (to remove sulfur compounds and some oxygenates).

In addition to the quality considerations already discussed, carbon dioxide safety is a key consideration for beverage industry technologists. Carbon dioxide is not usually considered to be a toxic gas in the generally accepted sense of the term (that is, poisonous) and is normally present in the atmosphere at a concentration of approximately 0.03% (300 ppm). Under normal circumstances, CO_2 acts upon vital functions in a number of ways, including respiratory stimulation, regulation of blood circulation, and acidity of body fluids. The concentration of CO_2 in the air affects all of these. High concentrations are dangerous upon extended exposure, due to increased breathing and heart rates, and a change in the body acidity. OSHA (Occupational Safety and Health Administration) establishes regulations governing the maximum concentration of CO_2 and the time-weighted average for exposure to CO_2. These regulations should be reviewed before installation of any CO_2 equipment, and the requirements fully met during operation and maintenance.

Because CO_2 is heavier than air, it may accumulate in low or confined areas. Adequate ventilation must be provided when CO_2 is discharged into the air. At lower levels where CO_2 may be concentrated, self-contained breathing apparatus or supplied-air respirators must be used. Filter-type masks should not be used. Appropriate warning signs should be affixed outside those areas where high concentrations of CO_2 gas may accumulate, and lock-out/tag-out procedures should be followed, as appropriate (Selz 1999).

19.3 SYRUP PREPARATION

Most carbonated beverage formulas begin with a "simple syrup", which is usually a simple combination of the nutritive sweetener (sucrose, HFCS, MIS) and treated water. In some cases, it may also contain some of the salts outlined in the specific beverage document, depending on the order of addition that is required. Once the sweetener is completely dissolved, and the simple syrup is a homogeneous batch, then the flavor and remaining components are added to form the "finished syrup". All simple syrups should be filtered before being pumped to the finished syrup blending/storage tanks.

19.3.1 Using Granulated Sucrose

Accurate weighing of granulated sugar is important. Granulated sugar is normally received in bulk form or in bags. Internationally, receipt in 50- or 100-pound jute or paper bags is not uncommon. It is extremely important that the sugar received by either means should be dry and free of lumps. Moist sugar creates two immediate and serious problems:

1. Moist sugar can have high microbial counts, much of which will be yeast. Yeast is a serious problem to carbonated beverages, because it can lead to fermentation and eventual spoilage of the finished product.
2. Moist sugar makes accurate measuring difficult, because the moisture content is being weighed, in addition to the sucrose solids. This makes final control of the batch difficult and inconsistent.

TABLE 19.6 Possible Brix Errors During Simple Syrup Production.

High Brix	Low Brix
Weighing error – excess sugar	Weighing error – short sugar
Faulty scale	Faulty scale
Instrument error	Not weighing sugar bags
Too little water	Too much water
	Instrument error
	Moist sugar

Source: Delonge (1994a).

Sugar in lumps will create difficulties in making simple syrup and will take longer to dissolve. Lump sugar is usually an indication that the sugar was not fully dried during refinery production or was stored improperly (Delonge 1994a). Bulk sugar systems should never be used where faced with wet or even slightly moist sugar. It will cause "bridging" (flow restriction) in silo storage and make effective handling impossible. It is critical that any bulk sugar supply is consistently dry and that the storage environment can be controlled to assure constant low humidity. Even the most modern silo can "bridge" when faced with a moisture problem.

Granulated sugar should always be added slowly into the treated water already measured into the tank. While sugar is being added, the tank agitator should be in constant operation. The agitation should continue until the sugar is completely dissolved. After the sugar has been completely dissolved, and the simple syrup has been filtered into the blending/storage tank, the syrup is checked for sugar content (Brix). Table 19.6 outlines intuitive, but useful, reasons for off-target Brix readings.

19.3.2 Using Liquid Sugars

There are three main types of liquid sugars that are used for syrup production, as discussed earlier: liquid sucrose, medium invert sugar, and high-fructose syrups. Making simple syrup from liquid sucrose is similar to the procedure employed when using granulated sugar. The first step is to check the Brix of the liquid sucrose to find out how much water must be added to the batch to bring the Brix of the simple syrup to the level required by the formula. Most companies' beverage documents include a table that specifies how much of the liquid sucrose and additional treated water should be added to the batch based on Brix. When liquid-sucrose supplies are received at the plant, they should be accompanied by an analysis sheet comparing the tank load against the company standards.

19.3.3 Medium Invert Sugar

Medium invert sugar is resistant to microbial spoilage when being transported from supplier to plant, and while in storage. However, good sanitation procedures are still required, as well as special precautions to prohibit secondary infection. When liquid invert shipments are received at the plant, they should be accompanied by an analysis sheet comparing the tank load against standards. The formula document should include a table that specifies how much of the sweetener and additional treated water should be added to the batch based on Brix and the percent inversion. When testing for Brix in MIS

samples, a correction factor must be used on refractometer readings to compensate for the nonsucrose solids as a result of inversion.

19.3.4 Using High-Fructose Syrups

For liquid sugars, in general, a sample should be taken before the sugar is accepted, and the analysis should confirm that the material is within standards. The installation, including receiving station, pumps, air blower/ultraviolet lamp, tanks and piping/fittings, should be of approved materials (stainless steel) and in accordance with the individual beverage company's design guidelines. High-fructose syrup is subject to crystallization, so storage temperatures should be controlled (generally maintained between 75°F/24°C and 85°F/29°C), by the use of indirect heating. The receiving station is a critical point and should be fully cleaned and hot sanitized before every delivery. As with MIS, when testing for Brix in high-fructose syrup samples, a correction factor must be used on refractometer readings to correct to true Brix and compensate for the nonsucrose solids.

No matter what type of nutritive sweetener is used, once the simple syrup has been correctly prepared in the mixing tank, it should be pumped through the syrup filter into the storage tank so that the other concentrate components may be added. Most simple syrups will be in a Brix range between 60 and 65, which makes them extremely susceptible to microbial spoilage, again, with yeast the most likely culprit. Be sure to recognize and respect any time constraints included in the syrup preparation instructions. For example, a general "rule of thumb" is that simple syrup should not be kept longer than four hours before converting it to finished syrup. If hot sugar processing is used, remember to atemperate the simple syrup to ambient temperature prior to the addition of concentrate. This will help minimize thermal degradation of the flavor oils. Also, it is very important to add the individual components in the specific order detailed in the syrup preparation instructions. Incorrect order of addition can lead to a variety of problems, including changes in viscosity, flavor degradation, nutrient breakdown, and precipitation of insoluble materials in the syrup tank.

19.4 CARBONATION

Earlier in this chapter, we discussed the history, theory, and principle of introducing CO_2 gas into water to produce a carbonated beverage. We also addressed the importance of the quality of this CO_2, as well as of the treated water used to dissolve it. In this section, we will discuss the practical aspects of carbonation control.

"Mix processing" refers to the process of combining the finished syrup, treated water, and CO_2 in the correct proportions to meet beverage specifications. In addition to the proportioning function, mix processing will usually incorporate deaeration, mixing, carbonating, and cooling, depending on the manufacturer's design and the type of products being handled. The design of mix-processing systems will vary from one manufacturer to another, incorporating the features that the manufacturer feels are advantageous to controlling production.

The primary function of the carbonating unit or the carbonator is to add CO_2 to the product. It must be carbonated to the level that, after filling and closing, results in a product within the standards for beverage carbonation. Some carbonating units incorporate cooling in the same tank or unit. The product can be slightly precarbonated with CO_2

injection and then exposed to a CO_2 atmosphere directly where cooling is in progress. Other systems separate the carbonating and cooling steps. The three most common forms of carbonating technology incorporate one or a combination of the following:

1. Conventional (atmospheric exposure) introduction;
2. CO_2 injection; or
3. CO_2 eduction.

The ability of water, or beverage, to absorb CO_2 gas, is largely dependent on the efficiency of the carbonating unit (24). Other factors that influence CO_2 absorption include

1. Product type;
2. Product temperature;
3. CO_2 pressure;
4. Time and contact surface area; and
5. Air content.

If the water temperature rises, the gas pressure must be increased if the same absorption of CO_2 is to be maintained. Conversely, if the temperature of the water or beverage entering the carbonating unit drops, the CO_2 becomes more soluble, and the pressure must be decreased to keep the volumes of carbonation within standards. Automatic CO_2 controls compensate for fluctuations in temperature, pressure, and flow. This allows the carbonating unit to produce a constant CO_2 gas absorption. Such controls are standard in modern processing units, which are available as basic units, or with computer interfaces to track the variation in product temperature, pressure, flow, and final CO_2 gas volumes absorbed during operating hours.

In many ways, this is a gross oversimplification of a process that, to this day, sometimes eludes strict control. Certainly, equipment has dramatically improved over the years, but loss of CO_2 remains a significant issue in terms of overall plant productivity. New membrane carbonation systems hold great promise for continuing this evolution, by helping to carbonate, at least in theory, more precisely and accurately than ever before. It has yet to be seen if these systems will endure the economic challenges, industry acceptance, and rigors of time.

19.5 FILLING, SEALING, AND PACKING

In the most fundamental terms, this section will address the introduction of the now freshly prepared and carbonated finished beverage into the package, and sealing it in a manner so as to preserve its integrity – simple in theory, sometimes challenging in application. The bottle-filling unit includes bottle handling/transfer components, a filling machine, and a capper/crowner.

The purpose of the filler is to fill returnable and nonreturnable bottles to a predetermined level. It should do this efficiently while minimizing foaming and deliver the bottle to a crowner or closure machine to be sealed, or, in the case of cans, to the lid seamer. A discussion of the design and engineering of filling machines is beyond the scope of this text, and is normally relegated to the specific operating manuals supplied by the respective equipment

TABLE 19.7 Problems Resulting from Foaming at the Filler

Quality	Economics/Operations.
Underfilled package	Impact on filling speed
Product residue on bottle	Loss of CO_2 and product
Incorrect CO_2 level	Increased BOD (biochemical oxygen demand) to the drain (sewer surcharge)
	Increased cost of clean-up

Source: Delonge (1994b).

vendor. Carbonated beverage fillers, to prevent the loss of CO_2 from the freshly carbonated beverage, must be counterpressured. The advantage in using CO_2 gas for counterpressure purposes at the filler bowl is to reduce product air content. With can fillers, this is possible because the counterpressure gas is normally purged from the can to the atmosphere as part of the filling process. Most bottle fillers presently in use vacate the counterpressure gas back into the filler bowl as the bottle is being filled. The empty bottle moving into the sealing position (at the filling valve) already contains air. Even if the counterpressure gas is CO_2, vacating this mixture (air and CO_2) back into the filler bowl ensures that the bowl will contain (predominantly) air. This can negate the advantage of CO_2 as a counterpressure gas, and actually be wasting CO_2 to the point of an economic disadvantage. In place of CO_2, air or nitrogen are sometimes used as the counterpressure gases.

Imagine what happens when a carbonated beverage is agitated, and then quickly uncapped. Sometimes, this same type of foaming that results can occur during filling. Foaming at the filler, even in small amounts, can cause a number of problems. Some of these deal with product quality, others with economics or plant operation, and are summarized in Table 19.7 (Delonge 1994b).

The cause(s) of foaming in a filling operation can range from a simple problem that can be corrected quickly, to one requiring extensive trial and error testing. Many times, the troubleshooting exercise requires a combination of technical skill, creativity, and experience. Some causes of foaming at the filler are summarized in Table 19.8. When the problem is a single valve, or occurs for a short period of time, it is usually easy to troubleshoot and correct. Ongoing foaming problems can be extremely difficult to correct. Manuals supplied by the manufacturer of the filler/mix processor usually address troubleshooting foaming problems in detail, and should be consulted. If the problem persists, contact the filler manufacturer.

One of the problems that can result from excessive foaming at the filler, aside from the poor aesthetics of "sticky" packages, is the formation of mold colonies on the external walls of the package. This might also be evident in the thread areas of bottles when the cap is removed. Proper sealing of the newly filled package is a critical step in the processing of carbonated soft drinks. The closure can be a variety of different types, including crimp-on metal crowns on glass bottles, screw-on metal or plastic caps on plastic bottles, or a seamed lid onto a can body. Each of these applications requires different equipment, but the over-riding objectives are the same:

1. Withstand the pressure from the CO_2 in this closed system;
2. Provide the consumer with a safely sealed product, and one with tamper evidence;
3. Prevent leakage of product out of the package; and
4. Help contribute to the visual appeal of the overall package.

TABLE 19.8 Some Causes of Foaming at the Filler.

Syrup overagitation	Glass quality and configuration
Dirty bottles	Excess air of dissolved oxygen in water
Dirty filler bowl	High syrup temperatures; warm bottles
Excessively carbonated product	Too high a liquid level in bowl
Warm product (inadequate refrigeration)	Frequent start/stop operation of filler (overagitation of product)
Line leaks (air introduction)	Carbo-cooler outlet valve not opening fully
Valve failure	Incorrect bowl pressure setting
Vent tube spreading rubber – wrong position or missing	Hot or contarminated CO_2
Vent tubes – scored, missing, loose, incorrect size	Damaged snift ferrule
Inadequate drainage after washing or rinsing	Rough transfer on A-frame
Particulates in water	Silicate or carbonate scale/deposits from water
Improper carbo-cooler operation	Worn valve liquid seal (skirt)
Inadequate carbo-cooler capacity, or operating beyond capacity	Dirty valve screens
Incorrect centering cup insert	Poor/leaking counterpressure seal
Product characteristics (more common in diets)	Worn pump seals on water, syrup, or beverage transfer pumps (air education)
Incorrect setting of valve operating/snift cams	
Bent valve operating leavers	

After proper application of the closure or lid, some beverage manufacturing plants pass the bottles and cans through a warmer, which is a tunnel of water sprays of carefully controlled temperature. The purpose is to bring the temperature of the filled packages (still cold from the chilled carbonated water introduced at the mix processor) up close to ambient. The main reason for this is to prevent excessive condensation, which can lead to problems, depending on the secondary and tertiary packagings that are used.

For example, in the United States and in many countries internationally, it is common to place bottles of carbonated beverage into rigid plastic crates for transport to a retail outlet. In these instances, warming is not usually needed, because the plastic crates are essentially inert, and allow for adequate air flow and ventilation of the product. Some products, however, perhaps because of a particular marketing promotion, will shrink-wrap multiple bottles together, then place them in a cardboard case box, and then stack them on a pallet that is stretch-wrapped for structural stacking integrity. In the second example, if the bottles were not warmed after filling, there is a high probability that the excess condensation would be trapped (by the shrink wrap), absorbed by the cardboard (presenting a mold risk), and then subjected to a "green-house effect" from the poor ventilation of the stretch wrap. It becomes quickly evident that a beverage producer's job is not complete simply because the product makes it safely to a sealed container!

19.6 QUALITY CONTROL AND ASSURANCE

In this section, we will distinguish quality "control" from quality "assurance" by having control refer to testing typically performed by the beverage plant, either immediately on-site or at a local contract laboratory. "Assurance" will refer to the subject of a broader, usually centrally managed program (for example, frequent testing of the

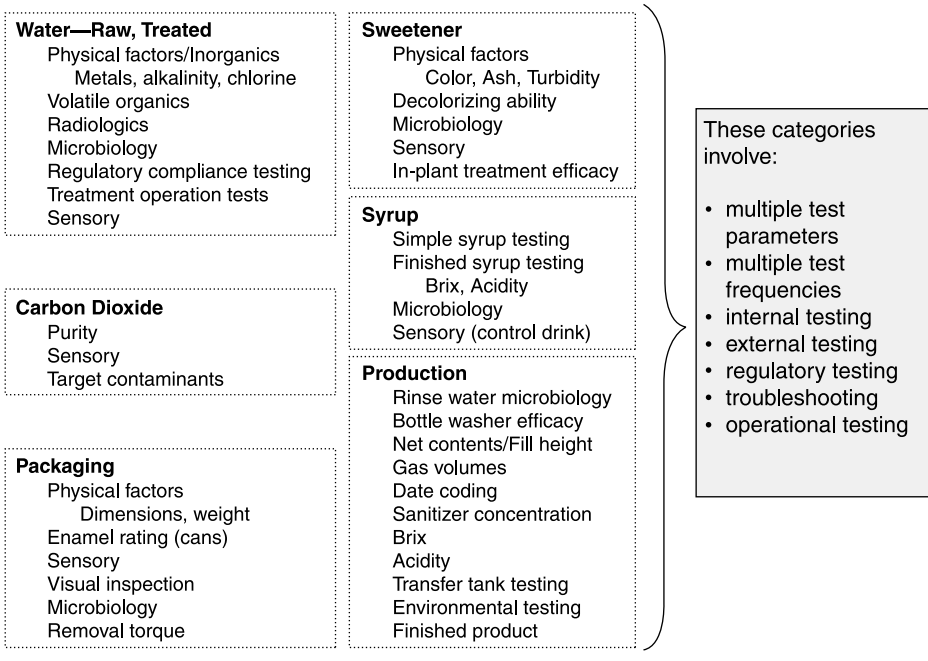

Figure 19.10 *Example of testing categories in a beverage plant quality scheme.*

product from the trade by a central corporate laboratory). Typically, the bulk of testing performed in a carbonated beverage facility falls under the category of quality control. Each company prescribes their own specific testing protocol, including the parameters to test, analytic test methods to apply, and frequency. In addition, a rigorous quality program would clearly outline the actions to be taken (and by whom) in the event that this testing demonstrates an out-of-specification situation.

Because there is no single protocol for all plants to follow, Figure 19.10 summarizes the major categories of testing to consider when evaluating a beverage plant's quality monitoring scheme. This list is by no means exhaustive, but it does provide an idea of how rigorous the monitoring and control in a beverage plant should be.

In addition to this quality control scheme, most larger beverage companies have developed formalized quality assurance schemes, which are usually under centralized corporate management. The programs generally include some auditing function to visit the production plants for compliance to standards and guidelines, and sampling of finished products from the trade. These programs vary in terms of their focus and rigor, but trade sampling provides perhaps the best representation of what the consumers in a particular market are receiving. From this perspective, the data obtained are of extreme value, and must be reviewed in concert with in-plant and external data, in order to provide the best overall picture of quality performance.

19.7 FINISHED PRODUCT

Low pH, high acidity, carbonation, and often ingredients that provide some natural antimicrobial activity (for example, d-limonene in citrus oils) – all of these combine to make

carbonated soft drinks a robust category of beverages. Of course, "robust" is a relative term, so as not to imply that carbonated beverages are completely immune to problems in the finished product. The formulas, however, go a long way in providing a margin of "designed" product safety.

In fact, for non-fruit-juice-containing carbonated beverages, the types of problems that are typically encountered in the trade are relatively few, and rarely, if ever, present a health or safety threat to the consumer. Microbiologically, we have already mentioned the possibility of having mold form where the overall moisture in the environment is not controlled. For example, remember the scenario of freshly filled bottles, moist with condensation, then shrink wrapped, palletized, and stretch wrapped. The resulting "green-house" effect could easily provide the necessary conditions in which mold could grow. In finished product, however, these beverages might contain a variety of organisms, but they will not remain viable under the conditions of the beverage. Only aciduric organisms can multiply, and these include some molds, yeasts, lactic acid bacteria, and acetic acid bacteria (Ray 2001). Of these, the clear majority of microbial problems are caused by spoilage yeast. This "spoilage" normally refers to any condition that affects the design appearance, flavor, or aroma of the product, and is usually a problem of esthetics where carbonated soft drinks are concerned.

In addition, as with any packaged products, the packaging materials can be the source of finished product problems. For example, misapplication of closures may occur, where removal torque is so high that consumers have difficulty opening the bottles. In areas of the world where returnable bottles are used, depending on their handling, they can become badly scuffed, presenting an unappealing look to the consumer.

Many problems with the finished product can be – and are – averted before the product ever leaves the beverage facility. This is due, in large part, to the diligent monitoring of the soft drink manufacturing process from beginning to end. We have already learned that the raw materials are held to high standards of quality upon receipt, and some–like water, sucrose, and carbon dioxide – are often further purified within the beverage plant itself. Then, these raw materials are combined into a finished syrup, and it is checked against standards of assembly and quality. This finished syrup is then diluted and carbonated, filled, then sealed to form the final beverage. The final product is tested chemically, microbially, and sensorially, to assure that it meets the highest standards of its trademarked brand.

This said, the summary above represents only a small portion of the quality systems that overarch most finished products, and which are clearly beyond the scope of this text. Suffice to say that many beverage companies begin to control quality as far back in the supply chain as possible – so far that some companies own their own citrus groves in order to strictly control the quality of the orange juice used in their orange-juice-containing carbonated beverages! In addition, as the principles of HACCP (Hazard Analysis and Critical Control Points) become more commonplace in the beverage industry, many bottlers and canners are voluntarily formulating their own HACCP plans to formalize the monitoring and control of their processes. All of this is done with a single, predominant end-goal in mind – to provide the consumer with a consistently high-quality, great-tasting, refreshing beverage.

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20

The Beer Brewing Process: Wort Production and Beer Fermentation

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20.1 Introduction 444
20.2 Brewhouse Operations 447
20.2.1 Introduction 447
20.2.2 Malt Milling 448
20.2.2.1 Dry Milling 448
20.2.2.2 Milling with Conditioning 448
20.2.2.3 Wet Milling 449
20.2.3 Mashing 449
20.2.3.1 Enzymatic Degradation Processes 449
20.2.3.2 Mashing Methods 451
20.2.4 Wort Separation (Lautering) 452
20.2.4.1 Mash Separation with a Lauter Tun 454
20.2.4.2 Wort Separation with a Mash Filter 454
20.2.5 Wort Boiling 455
20.2.5.1 Wort Boiling Objectives 456
20.2.5.2 Energy Saving During Wort Boiling 459
20.2.5.3 Wort Boiling Systems 461

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

20.2.6 Wort Clarification 466
20.2.6.1 Spent Hops Removal 466
20.2.6.2 Hot Trub Removal 467
20.3 Beer Fermentation 467
20.3.1 Biochemistry of the Primary Beer Fermentation 467
20.3.1.1 Carbohydrate Metabolism: Ethanol Production 467
20.3.1.2 Metabolism of Bioflavoring Byproducts 471
20.3.2 Biochemistry of Secondary Beer Fermentation 479
20.3.2.1 Vicinal Diketones 479
20.3.2.2 Hydrogen Sulfide 480
20.3.2.3 Acetaldehyde 481
20.3.2.4 Development of Palate Fullness 481
20.3.3 Beer Fermentation Technology 482
20.3.3.1 Batch Fermentation Technology 482
20.3.3.2 Beer Fermentation Using Immobilized Cell Technology 484
References 490

20.1 INTRODUCTION

The production of alcoholic beverages is as old as history. Wine may have an archeological record going back more than 7.5 thousand years, with the early suspected wine residues dating from early to mid-fifth millennium B.C. (McGovern and others 1996). Clear evidence of intentional winemaking first appears in the representations of wine presses that date back to the reign of Udimu in Egypt, some 5000 years ago. The direct fermentation of fruit juices, such as that of grape, had doubtlessly taken place for many thousands of years before early thinking man developed beer brewing and, probably coincidentally, bread baking (Hardwick 1995). The oldest historical evidence of formal brewing dates back to about 6000 B.C. in ancient Babylonia: a piece of pottery found there, shows workers either stirring or skimming a brewing vat.

Nowadays, alcoholic beverage production represents a significant contribution to the economies of many countries. The most important beverages today are beer, wine, distilled spirits, cider, sake, and liqueurs (Lea and Piggott 1995). In Belgium ("the beer paradise"), beer is the most important alcoholic beverage, although beer consumption has declined in the last 40 years: from 11,096,717 hL in 1965 to 9,702,619 hL in 2004 (Anon. 2005a). In this time frame, wine consumption doubled from 1,059,964 to 2,471,388 hL. Another trend is the spectacular increase in water and soft drinks consumption (from 5,215,056 to 26,395,000 hL).

The principal raw materials used to brew beer are water, malted barley, hops, and yeast. The brewing process involves extracting and breaking down the carbohydrate from the malted barley to make a sugar solution (called "wort"), which also contains essential nutrients for yeast growth, and using this as a source of nutrients for "anaerobic" yeast growth. During yeast fermentation, simple sugars are consumed, releasing energy and producing ethanol and other flavoring metabolic byproducts. The major biological changes that occur in the brewing process are catalyzed by naturally produced enzymes from barley (during malting) and yeast. The rest of the brewing process largely involves heat exchange, separation, and clarification, which only produces minor changes in chemical

composition when compared to the enzyme-catalyzed reactions. Barley is able to produce all the enzymes that are needed to degrade starch, β-glucan, pentosans, lipids, and proteins, which are the major compounds of interest to the brewer. An overview of the brewing process is shown in Figure 20.1, where the input and output flows are also indicated. Table 20.1 gives a more detailed explanation of each step in the process.

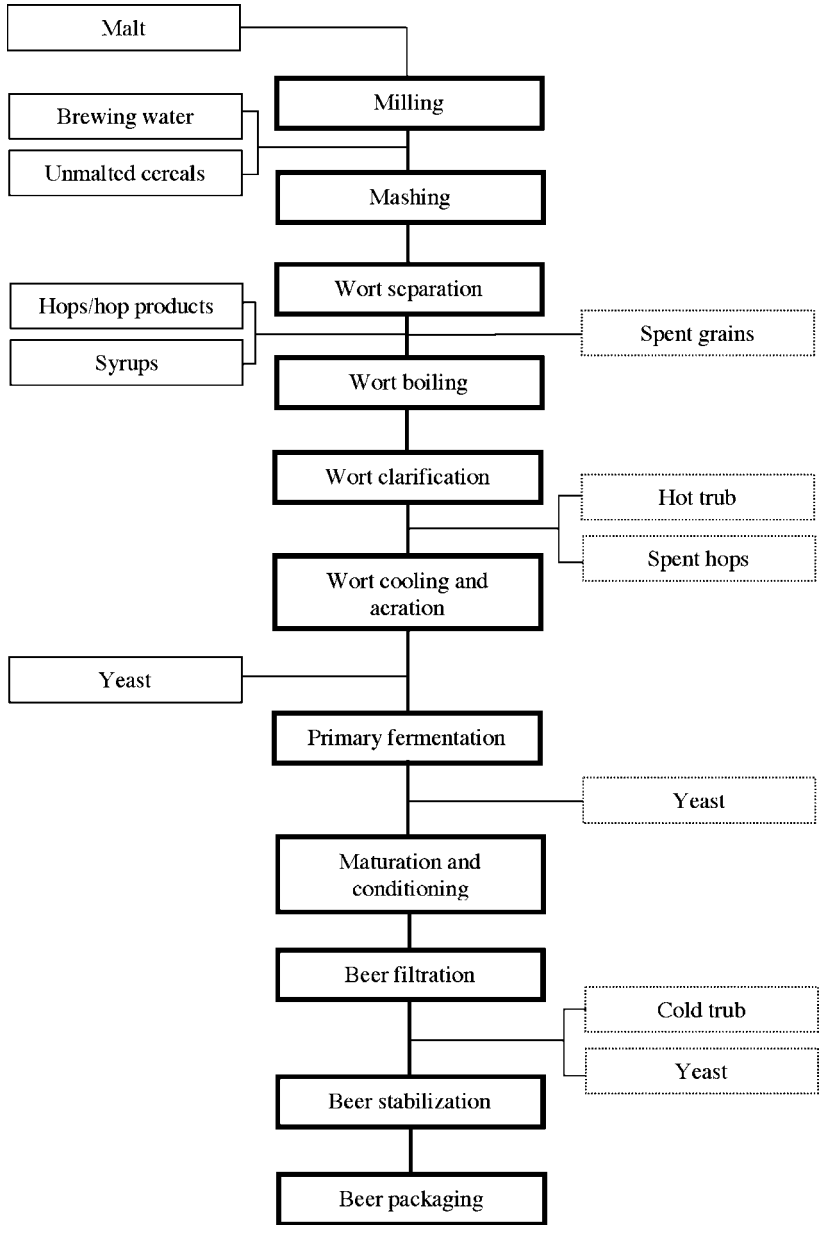

Figure 20.1 *Schematic overview of the brewing process (input flows are indicated on the left side and output flows on the right side).*

TABLE 20.1 Overview of the Brewing Processing Steps: From Barley to Beer.

Process	Action	Objectives	Time	Temperature (°C)
Malting				
Steeping	Moistening and aeration of barley	Preparation for the germination process	48 h	12–22
Germination	Barley germination	Enzyme production, chemical structure modification	3–5 days	22
Kilning	Kilning of the green malt	Ending of germination and modification, production of flavoring and coloring substances	24–48 h	22–110
Milling	Grain crushing without disintegrating the husks	Enzyme release and increase of surface area	1–2 h	22
Mashing + wort separation	Addition of warm/hot water	Stimulation of enzyme action, extraction and dissolution of compounds, wort filtration, to obtain the desired fermentable extract as quickly as possible	1–2 h	30–72
Wort boiling	Boiling of wort and hops	Extraction and isomerization of hop components, hot break formation, wort sterilization, enzyme inactivation, formation of reducing, aromatic and coloring compounds, removal of undesired volatile aroma compounds, wort acidification, evaporation of water	0.5–1.5 h	>98
Wort clarification	Sedimentation or centrifugation	Removal of spent hops, clarification (whirlpool, centrifuge, settling tank)	<1 h	100–80
Wort cooling and aeration	Use of heat exchanger, injection of air bubbles	Preparing the wort for yeast growth	<1 h	12–18
Fermentation	Adding yeast, controlling the specific gravity, removal of yeast	Production of green beer, to obtain yeast for subsequent fermentations, carbon dioxide recovery	2–7 days	12–22 (ale) 4–15 (lager)
Maturation and conditioning	Beer storage in oxygen-free tank, beer cooling, adding processing aids	Beer maturation, adjustment of the taste, adjustment of CO_2 content, sedimentation of yeast and cold trub, beer stabilization	7–21 h	−1–0
Beer clarification	Centrifugation, filtration	Removal of yeast and cold trub	1–2 h	−1–0
Biological stabilization	Pasteurization of sterile filtration	Killing or removing of microorganisms	1–2 h	62–72 (past.) −1–0 (filtr.)
Packaging	Filling of bottles, cans, casks, and kegs; pasteurization of small volumes in packings	Production of packaged beer according to specifications	0.5–1.5 h	−1 to room temperature

20.2 BREWHOUSE OPERATIONS

20.2.1 Introduction

The technology of brewing (wort production) can be classified into six unit processes: grinding of malt and adjuncts, mashing, wort filtration, wort boiling, wort clarification, and wort cooling and aeration. Figure 20.2 shows schematically the different unit operations in the brewhouse. In a classical brewhouse, the malt is milled and the grist is mixed with brewing water ("mashing in"). Mashing can be performed using the infusion method or the decoction method. In the case of using adjuncts with a high gelatination temperature, the use of an adjunct cooker is necessary. In the filter vessel or the mash filter, the liquid extract ("the wort") is separated from the insoluble material (called "the spent grains"). Next, wort is boiled in a boiling vessel. After boiling, the hot trub is removed by a whirlpool (or a sedimentation tank, or a centrifuge), and cooled using a plate heat exchanger until fermentation temperature and aerated.

The aim of the brewhouse operations is to make a malt (and adjunct) extract as efficiently as possible. During the brewing process a few chemical transformation processes occur, that is, the oxidation of polyphenols, the formation of lipid–protein complexes and the precipitation of proteins. The nitrogen and carbohydrate composition of the wort depends on the enzyme-to-substrate ratio, that is, the ratios α- and β-amylases/starch and endo-peptidases/proteins. These ratios can be adjusted by the following techniques: the use of substrates without enzyme activity (adjuncts) reduces the amount of nitrogen in wort, thermal destruction of enzymes by boiling during decoction mashing, adjusting the pH to influence the activity of the enzymes, adjusting the malt/water ratio, which has an influence on the activity of certain enzymes during mashing (e.g., proteolytic proteins are more active in a concentrated mash).

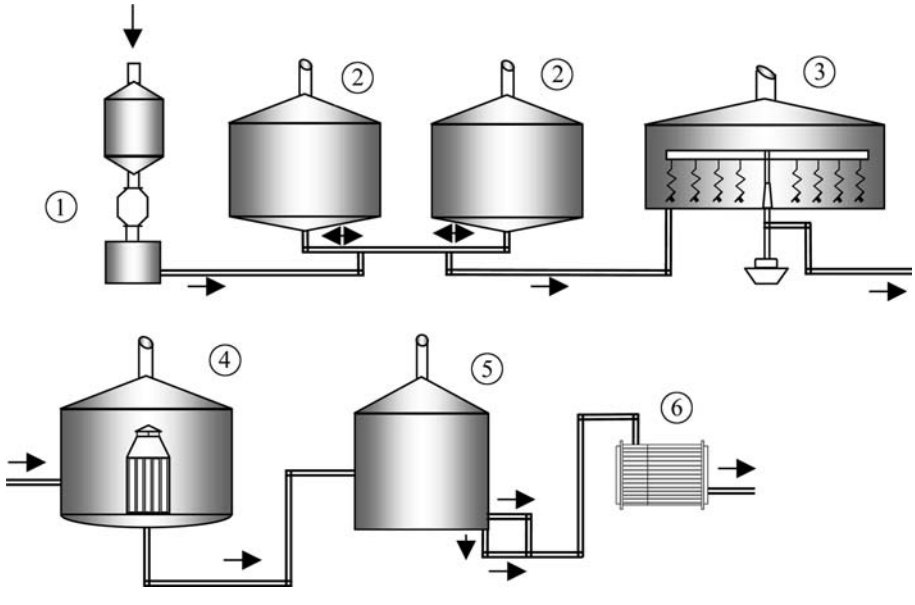

Figure 20.2 *Wort production in a classical brewhouse: 1. malt grinding, 2. mash vessels (decoction mashing), 3. wort filtration in a filter vessel, 4. wort boiling, 5. wort clarification in a Whirlpool, 6. wort cooling (heat exchanger).*

20.2.2 Malt Milling

By milling malt, the starchy endosperm will become better accessible for the malt enzymes and this will improve the extraction process. The selected milling technique depends on the methods of mashing and separation that are used. Milling should be performed in conditions that preserve the structure of the husks when a lauter or mash tun is used, as in this case the husks are needed to build up the filtration bed. The husks are more elastic and will be less damaged during milling when they are more humid. Therefore, the malt is usually made humid (called "conditioned" or "wet" milling) before it is milled. Dry or wet milling is performed in a roller mill. When modern thin-bed mash filters are used, filtration is performed using a filter cloth and intact husks are not needed. For this case, malt is milled very finely using a hammer mill. In modern mills, the milling is performed in the presence of deaerated water to avoid oxygen take-up, using a disc (Biche and others 1999; De Brackeleire and others 2000) or rotor–stator (Anon. 2004) system.

20.2.2.1 Dry Milling. Dry milling is the most popular milling technique (Kunze 1999; N.N. 1999). The degree of malt modification, the position and the type of roll surface (smooth or fluted) determine the particle distribution. Roller and hammer mills can be used for dry grinding.

Rolling mills operate by passing the malt through the narrow gap between pairs of closely spaced rotating rolls. Various roll arrangements are possible: 2, 3, 4, 5, or 6 rolls (Table 20.2). Most designs have 2 or 3 pairs with an intermediate separation stage or stages. This enables the fine material, which does not need further milling, and the husks to be separated from coarser material, which does need further milling. Oscillating (frequency of 6–12 Hz) sieves perform the separation steps. The sieves are equipped with a large number of rubber balls or other proprietary sieve cleaners. The mill with 6 rolls gives the best results and is used most frequently.

In a hammer mill, the malt is reduced in size to fine grist. A large rotating drum on which small hammers are mounted, propel the dry cereal against a striking plate. The produced fine grist is sieved at the base of the drum. The length and number of hammers, screen perforation size, and rotation speed influence the particle size of the grist, capacity, and power consumption and heat generation.

20.2.2.2 Milling with Conditioning. In this process, the malt is moisturized with cold or hot water, or steam. Moist husks are more pliable and will be less damaged in a roll mill. The temperature inside the kernel may not exceed 40°C to avoid damage to

TABLE 20.2 Types of Roller Mills.

Roll Arrangement	Capacity (kg/h/mm)	Uses	Suitability
2 rolls without sieves	1.5–2.5	Malt and cereals	Small "pub breweries" only
3 rolls without sieves	2.0–3.0	Malt and cereals	Small "pub breweries" only
4 rolls with or without sieves	2.0–6.0	Malt	Lauter tuns and mash filters
5 rolls with sieves	2.0–6.0	Malt	Lauter tuns and mash filters
6 rolls with sieves	2.0–10.0	Malt	Lauter tuns
6 rolls with sieves	1.5–8.0	Malt	Mash filters

Source: N.N. (1999).

the enzymes. The total water content increases by about 0.7%. This corresponds to an increase in water content of the husks of 1.5–1.7% and 0.3–0.5% of the endosperm. The effect of the conditioning results in an increase of the husk volume of about 10–20%, an easier separation of the grist and husks fraction, an increase in filtration rate in a lauter tun, an increased yield and attenuation, and a faster starch degradation. Conditioning can be performed using a conditioning screw where the malt is wetted when it passes through a screw conveyor.

20.2.2.3 Wet Milling. The moisture content of the husks can be raised to 20% by keeping the endosperm nearly dry in a hot water conditioning mill. This process is referred to "hot water conditioning" (Anon. 1999). In this case, the endosperm is squeezed from the husk through a single pair of rolls. After milling, mashing water is immediately added. The quantity of conditioning water depends on the malt quality, degree of modification, and achieved steeping degree.

In the "steep conditioning" process, the moisture content is raised to a much greater extent and becomes part of the mashing process. Typically, steeping lasts between 10 and 30 min at a temperature between 30 and 50°C. The temperature and duration depend on the modification and the moisture content of the incoming malt. The moisture content of the malt increases to 25–30%. The total milling and mashing-in time is in the range 30–45 min.

20.2.3 Mashing

Mashing starts with mixing the grist and brewing water (called "mashing-in"). Hydration enables the malt enzymes to become active. Today, mashing-in is part of the milling process, where it is performed in the milling equipment. Formerly, a "premasher" ("foremasher" or "grist hydrator") mixed the grist and the water on their way into the mash vessel. Deaerated water is used to minimize oxygen uptake. Typically, 2–4 hL water is used for 100 kg malt, depending on the selected brewing method and density of the produced beer.

During mashing, the malt content is solubilized by making use of the enzymes of the malt and the extract is obtained (Table 20.3). By manipulating the temperature profile and the duration of rest periods at specific temperatures, the brewer is able to influence the composition and efficiency with which the malt is extracted. The mashing operation will influence the alcohol content of the beer, the concentration of unfermented sugars in the beer, the peptide and amino acid profiles of the wort, the yeast nutrient concentration, the buffering capacity and pH of the wort and beer, the β-glucan content of the beer, and some beer physical properties such as foam, color, and clarity (Rehberger and Luther 1995).

20.2.3.1 Enzymatic Degradation Processes

Starch Degradation. Starch is degradated by α- and β-amylase, limit dextrinase, maltase, and saccharase (Table 20.3). Gelatinized starch is needed for β-amylase in order to degrade starch efficiently. Barley starch gelatinizes in the presence of amylases at 60°C. Rice starch gelatinizes at 80–85°C and thus needs to be gelatinized before it is added to the mash. Pregelatinization is performed in an adjunct cooker. The action of the α-amylases will result in a decreased viscosity of the gelatinized starch

TABLE 20.3 Active Enzymes During Mashing.

Enzyme	Optimum pH	Optimum Temperature (°C)	Inactivation Temperature (°C)	Hydrolysis Reaction	Product
			Starch Degradation		
β−Amylase	5.4−5.6	60−65	70	α-1,4 bond at the nonreducing end of starch	Maltose
α-Amylase	5.5−5.8	70−75	80	α-1,4 bond in starch	Dextrin
Limit dextrinase	5.1−5.5	55−60	65−70	α-1,6 bond in starch	Dextrin (straight chain)
Maltase	6.0	35−40	40	Maltose	2 Glucose
Saccharase	5.5	50	55−67	Saccharose (sucrose)	Glucose + fructose
			Protein Degradation		
Endo-peptidase	5.0−5.5	40−60	60−80	Peptide bond inside chain	Short peptides
Carboxy-peptidase	4.8−5.6	50−60	70	Peptide bond at carboxy end	Amino acids
Amino peptidase	7.0−7.3	40−45	50−55	Peptide bond at amino end	Amino acids
Dipeptidase	8.0−8.8	40−45	50	Dipeptide	2 Amino acids
			Cell Wall Degradation		
Endo-β-1,4-glucanase	4.5−5.0	40−45	50−55	β-1,4-bond	β-Glucan with low MW
Endo-β-1,3-glucanase	4.6−5.5	60	70	β-1,3-bond	β-Glucane with low MW
β-Glucan solubilase	6.3−7.0	62−70	73	Bond between β-glucane and protein	β-Glucan with high MW
Endo-xylanase	5.0	45		Pentosan: xylan chain	Xylose
Exo-xylanase	5.0	45		Pentosan: xylan chain	Xylose
Arabinosidase	4.6−4.7	40−50	60	Pentosan: araboxylan chain	Arabinose
			Others		
Phosphatase	5.0	50−53	60	Organic phosphate	Phosphoric acid
Lipase	6.8	35−40	70	Lipids	Fatty acids

Source: Narziss 1992; Heyse 1995; O'Rourke 1995; Kunze 1999; Narziss 2005.

("liquefaction"). The complete degradation of starch to maltose and dextrines by amylases is called saccharification. Starch breakdown must be monitored because residues of undegraded starch and dextrins cause starch hazes in beer. Starch degradation can be easily monitored by checking the color of a mixture of a mash sample and an iodine solution (iodine test): a positive starch is indicated by a blue/black color. α-Amylase is active during malting (5−10% starch degradation), but is much more active on gelatinized starch. It needs Ca^{2+} as a cofactor, and does not produce maltose during degradation. β-Amylase is present during malting, but is not active. It produces maltose, β-dextrins, glucose, and maltotriose from amylose and amylopectin. Limit dextrinase breaks the

1,6-bonds in small, branched dextrins. Its presence in malt is limited. Maltase hydrolyses maltose into glucose molecules. However, it is not active above 40°C.

Cell Wall Degradation. Cell walls are essentially composed of β-glucan and hemicellulose. Cell wall degradation starts during malting and continues during mashing by endo-β-glucanases, β-glucan solubilase, and endo-xylanase (Table 20.3). An insufficient degration of high MW β-glucan molecules results in a high viscosity and can give problems during wort and beer filtration (Meilgaard 1976). The extent of the problems can be diminished by the extent of malt modification or adding commercial β-glucanase preparations (O'Rourke 1996). An insufficient degradation of pentosans (hemicellulose) can result in filtration and haze problems (Coote and Kirsop 1976; Viëtor and other 1991; Cach and Annemüller 1995; Han and Schwarz 1996; Stewart and others 1998). Pentosans (also called arabinoxylans) can be degraded to arabinose and xylose by xylan solubilase, endo- and exo-xylanase, arabinosidase, and xylobiase (Fincher and Stone 1993; Debyser and others 1997, 1998).

Protein Degradation. Protein degradation products influence fermentation and beer flavor (lower molecular weight (MW) degradation products), palate fullness (amino acids and higher MW degradation products), color (Maillard reaction), and beer foam (higher MW degradation products). A too extensive proteolysis gives bad foam, too dark color, poor palate fullness, but a good colloidal stability. During malting and mashing, 35–40% of the total protein content is degraded. During malting 60% of the amino acids are produced, the rest during mashing. Carboxypeptidases play an important role in the production of amino acids, due to their optimal pH and temperature conditions (Table 20.3). The enzymatic breakdown of proteins occurs predominantly at 45–55°C, but does not stop even at higher temperatures. With a rest period at 45°C, more lower MW products are formed. It is necessary to supply yeast with sufficient α-amino acids for growth and metabolism. The α-amino nitrogen concentration must be at least 20 mg/100 ml wort (Kunze 1999). Worts from normally modified malts always contain sufficient α-amino acids. At 55°C, more high-MW compounds are produced. When mashing with well-modified malts, the extent of proteolysis is much less than that derived from the malting process (Lewis and others 1992). A long rest at 50°C always results in a poor foam (Kunze 1999).

20.2.3.2 Mashing Methods. Mashing is performed in a mashing vessel (also called mashing tun, mash mixer, or mash converter). Nowadays, it is constructed in stainless steel and heated with steam through semicircular pipes welded on the tun bottom and body. An agitator is used to ensure efficient and homogeneous mixing during mashing.

Possible rest periods, which are chosen at the temperature optima of the enzymes, are at the following temperatures:

- 45–50°C for proteolysis and β-glucan degradation;
- 62–65°C for maltose production (β-amylase);
- 70–75°C for saccharification (α-amylase); and
- 78°C as the final mash temperature to inactivate the carbohydrate enzymes and fix the amount of fermentable sugars.

Mashing methods can be classified into two types of processes: infusion and decoction processes.

Infusion Method. In infusion processes, the entire mash is heated up (with appropriate rest periods) to the final mashing temperature. Infusion methods can be classified as increasing temperature (German infusion method) and decreasing temperature (English infusion method) infusion processes (Fig. 20.3 shows some examples). A classical German infusion method starts with a rest period at 45–50°C (proteolysis) for 30 min. Next, the temperature is raised to 62–65°C and kept at this temperature for 30–45 min (β-amylase). The next rest period is at 70–75°C until complete saccharification (α-amylase). The process ends at 78°C. In the English infusion method, the temperature is initially raised by adding hot water to the mash. This method requires well-modified malt, because a part of the active enzymes are destroyed by the addition of hot water. An alternative English method is to perform the conversion processes at a single temperature, usually 63–65°C ("isothermal infusion mashing"). These English infusion methods are used for the production of ales (top fermentation), where both mashing and wort separation take place in the same vessel (O'Rourke 1996).

The advantages of the infusion method are that this process can be easily automated and controlled, and energy consumption is 20–30% lower than for the decoction method (Kunze 1999). Disadvantages are the rather worse iodine reaction and the possibly rather lower brewhouse yield.

Decoction Method. In decoction processes, the temperature is increased by moving part of the mash from the mash converter to the mash cooker where it is boiled (possibly also with carbohydrate rest periods in the mash cooker). By pumping it back to the remainder of the mash in the mash converter, the temperature of the total mash is increased to the next higher rest temperature.

The decoction method is traditionally used in Germany for the production of lager beer. Depending on the number of boiled mashes, decoction methods can be classified as single-, two-, and three-mash processes. Today, only the single- or two-mash processes are used. The three-mash process consumes a lot of energy and is only used for the production of some special beers. A typical two-mash scheme is shown in Figure 20.3(*c*).

The removal and boiling of the boiled mashes have the following effects (Kunze 1999):

- Less protein breakdown in the boiled mash because of more rapid heating;
- More extensive gelatinization and saccharification of the starch;
- Increased extraction of the husks;
- Increased formation of melanoidins (Maillard reaction);
- Increased removal of dimethyl sulfide (DMS);
- Reduced amounts of active enzymes in the total mash; and
- Possibly a higher brewhouse yield.

20.2.4 Wort Separation (Lautering)

During the lautering process (also called "wort separation" or "mash separation") the undissolved substances are separated from the wort. The insoluble part (spent grains) consists of the husks, the seedlings, and other insoluble material. Wort separation is a filtration process. As much of the extract as possible should be recovered during

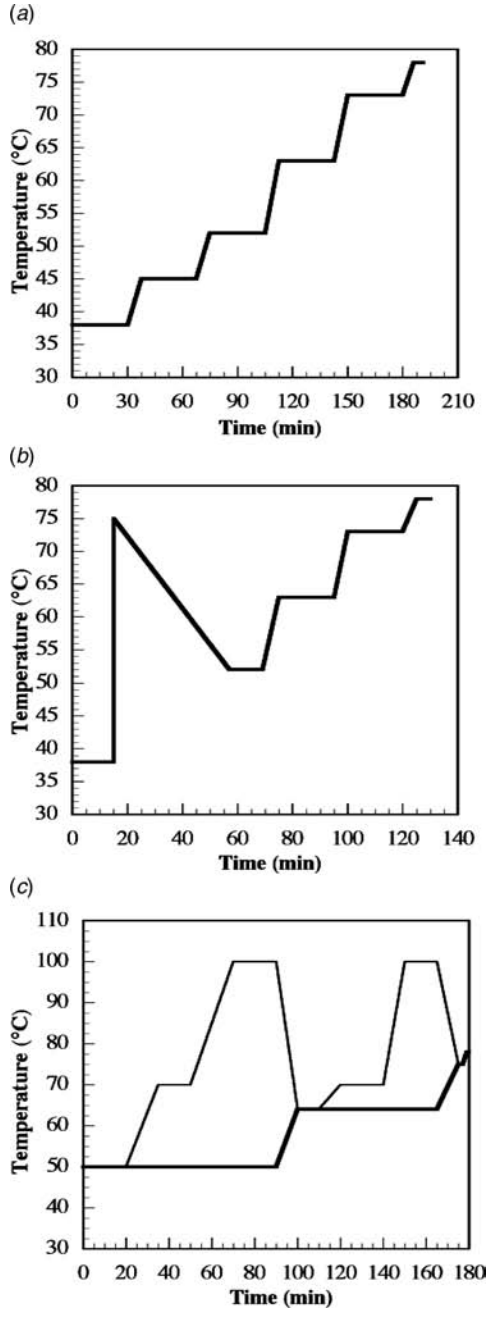

Figure 20.3 *Mashing schemes: (a) increasing infusion mashing, (b) decreasing infusion mashing, (c) two mash decoction process.*

lautering. The extraction efficiency is measured as "extract yield", which is the ratio of the mass of extract to the mass of malt or malt and adjunct (Rehberger and Luther 1995).

The filtration can be performed in a lauter tun or a mash filter. Developments in wort separation, which have been introduced over the last 15–20 years, are summarized in Table 20.4.

20.2.4.1 Mash Separation with a Lauter Tun. Today, lauter tuns are made of chrome–nickel steel and thermally insulated. A spent grain filtration layer is formed on a welded wedge wire false bottom of chrome–nickel steel, which is fixed 10–20 mm above the tun bottom. Up to 15% of the false bottom surface is composed of gaps. The lauter tun is initially filled with hot (78–80°C) water and the mash is next fed from the bottom to keep the oxygen uptake as low as possible. The loading depends on the quality of the raw material: 170–210 kg/m^2 in the case of conditioned milling and 200–280 kg/m^2 in the case of steep conditioned milling (Kunze 1999; Narziss 2005). At the bottom of the tun are runoff ports for collection of the liquid from the mash.

After filling the tun, the spent grains settle for 20–30 min and form the filtration layer (height of ~30–40 cm; 60–70 cm in the case of wet milling). A tun bottom dough, which consists of particles that have passed through, collects at first between the tun bottom and the false bottom. It is pumped back with the first cloudy wort until the withdrawn wort is clear. The first wort passes through the spent grains and is thereby filtered. Rakes cut the filtration layer in order to keep the pressure difference over the layer as low as possible. The first wort is allowed to run off until the spent grains become visible. Next, sparge water (75–78°C) is used to wash the mash and to displace the wort downwards. When the filtration operation is complete, the remaining water is drained to sewer and the spent grains are discharged.

20.2.4.2 Wort Separation with a Mash Filter. These filters perform wort separation in a thin bed (6–7 cm) process. Their basic design evolved from early inefficient units that produced high wort turbidity and solids to modern (on the market since the beginning of the

TABLE 20.4 Developments in Wort Separation.

Aim	Development
Extended beer-keeping quality	• Minimize oxygen pick up • Avoid excessive shear in stirring and transfer of mash
Increase collection gravity	• Reduce volume of lauter bottom chamber
Improve wort clarity	• Control milling procedures • Recirculation of initial worts • Sparging from inside to outside of thin compressed grain bed with new mash filter
Achieve higher extract yield	• Reuse of covered weak worts • Finer milling with new mash filters
Shorten cycle time	• Introduce new lauter knife designs and ensure balanced runoff from tun floor • Use thin bed in mash filters

Source: Daoud (1990).

1990s) fully automated units that produce up to 14 quality brews per day. A successful, modern mash filter is the Meura 2001 from the company Meura (Tournai, Belgium) (Jones 1992; Melis and Eyben 1992; Anon. 2005b). This filter consists of a fixed end frame and rear caisson, together with the side support beams form the basic framework of the machine. Within the frame is a hydraulic ram, which moves the mobile end into the open or closed position. In the closed position this compresses the plate pack composed of alternately placed polypropylene plates (polypropylene filter cloths on both sides through which the wort can flow) and polypropylene frames (elastomer membranes on both sides to compress the mash through air injection). The frames are connected via pneumatic tubing to a main compressed air header above the machine. The number of plates and frames installed and thus the length of the entire filter is determined by the required raw material capacity.

The Meura 2001 filter travels through three operation phases:

1. Filling–filtration;
2. Precompressing–sparging; and
3. Compression phase (Anon. 2005b).

During the first phase, the mash enters the filter from below and is distributed over the various chambers. When the filter is filled and the bed has started to form on the cloths, the wort will start to run bright. Once all the mash has been transferred, the transfer line is rinsed and the filter beds are precompressed. After filtration, more than 80% of the soluble sugars can be recovered. As a result of precompression and relatively "slow" sparging, the sparge rate can be as low as 2.2 hL/100 kg malt grist. Precompression of the filter beds, prior to sparging, helps to recompose the bed and makes it possible to recover most of the extract with heavy worts. In a final phase, dewatering is achieved by mechanical compression of the cakes by inflating the elasticated membranes.

20.2.5 Wort Boiling

Wort boiling is a complex process during which a wide range of chemical, physicochemical, physical, and biochemical reactions occur. Wort boiling is the most energy-intensive stage in the brewing process. Brewhouses that are equipped with old technology require a lot of energy to heat up the wort and are characterized by a long-lasting boiling process. Alternative wort-boiling technologies, such as low-pressure boiling and high-temperature wort boiling, have been studied in detail during the last decades, with a focus on the reduction of primary energy consumption. Recently, new boiling systems have been developed and commercialized (Willaert and Baron 2005). The new systems reduce energy consumption still further and are all characterized by exerting a low thermal stress on the wort during boiling. A low thermal load has a positive influence on the sensorial and foam characteristics of the produced beer. The thermal stress can be quantitatively assessed by measuring the colour, thiobarbituric number or the concentration of high-temperature indicators (Manger 2000). The thermal load can be reduced by (combining) the following measures:

- Application of the infusion mashing technique (instead of decoction mashing);
- Reduction of the heating time of the wort before boiling;
- Reduction of the boiling time;

- Reduction of the temperature during boiling and high-temperature holding periods;
- Reduction of the filling and rest time of the whirlpool;
- Reduction of the wort cooling time.

20.2.5.1 Wort Boiling Objectives. The wort has to be boiled just before it is aerated and used as the nutrient broth for alcoholic fermentation by the yeast cells. The wort boiling process aims at several objectives (see Table 20.5), as in the following.

Extraction and Isomerization of Hop Components. Bitter hops – hop cones, pellets type 90 or 45 – are added at the start of the boiling process. It is necessary to sustain a high temperature over a certain time to obtain a high isomerization yield of the α-acids. The isomerization yield depends on:

- The nature of the isohumulone (cohumulone gives the best yield);
- The duration of the boiling;
- The pH (a higher pH gives a higher yield, but the obtained bitterness at lower pH is more balanced and finer, and thus preferred);
- The humulone concentration (decreasing yield upon increasing concentration);
- Precipitation of isohumulone with the hot break;
- The use of more efficient extraction procedures (such as the use of higher temperatures);
- Size of the hop fragments (extraction rate is higher for milled hop cones or pellets).

Nowadays, the brewer can choose between different hop products. Some hop products, such as isomerized pellets, isomerized kettle or hop extracts, and reduced isomerized α-acids (i.e., tetra hydro-iso-α-acids), are already isomerized before they are used and

TABLE 20.5 Objectives of the Wort Boiling Process.

Objective	Influencing Parameters
Extraction of α-acid	Temperature, fragment size
Isomerization of α-acid	Temperature, boiling time, pH, humulone concentration
Coagulation of proteins (hot break formation)	Boiling time, nature and manner of boiling, heating system, copper shape and wort flow configuration, temperature of heating medium, wort composition (malt modification, kilning temperature, mashing temperature, pH)
Wort sterilization and inactivation of enzymes to fix the wort composition	Boiling time, temperature
Formation of reducing and aromatic compounds (Maillard reaction)	Water content, pH, oxygen, concentration, temperature, reaction (boiling) time
Formation of coloring substances	pH, parameters influencing the Maillard reaction
Removal of undesired volatile aroma compounds	Temperature, boiling time, pH, evaporation rate
Acidification of the wort	Hop addition, intensity of the Maillard reaction, alkaline phosphates and Ca^{2+} and Mg^{2+} content, malt type
Evaporation of water	Temperature, boiling time

Source: Narziss (1978), Hough and others (1982), Narziss and others (1982a), Miedoner (1986), Enari (1991), Narziss (1992), Kunze (1999).

need not be added at the wort copper, but can be applied at the end of the brewing process. By using these new hop products, it is no longer necessary to keep the wort over a "long" period at a high temperature.

Hot Break Formation. During boiling, two types of compounds are formed:

1. Compounds consisting of proteins and polyphenols, and compounds consisting of proteins and oxidized polyphenols that are insoluble in hot wort and precipitate as hot break;
2. Compounds formed from protein degradation products and polyphenols, which remain in solution during boiling and only precipitate as cold break when the wort is cooled (Kunze 1999).

Polyphenols are not directly involved in protein coagulation because protein–polyphenol complexes are based on hydrogen bondings, which have only a very weak binding energy at boiling temperatures and are unstable under these conditions (Miedaner 1986; Narziss 1992). Hot break formation is encouraged by longer boiling times, vigorous movement of the boiling wort (which improves the reaction between proteins and polyphenols), and a low pH, because the coagulation is best accomplished at the isoelectric point of the proteins. To obtain a sufficient coagulation, a pH of 5.2 is recommended (Narziss 1992; Kunze 1999). The isoelectric pH of some proteins – such as β-glubulins, δ- and ε-hordein – is very low (pH 4.9) and cannot always be realized during wort boiling. The removal of high-MW coagulable proteins is very important for the composition and the quality of the finished beer. Insufficient coagulation and removal result in a poor fermentation because the transport of substrates to and products from the yeast cells is hindered by the hot break adsorbed on the yeast cell walls. This leads to an insufficient pH drop during the primary fermentation and therefore to an incomplete elimination of proteins during the main fermentation, followed by a poor clarification during storage. This can result in a beer with a harsh bitterness ("protein bitterness") and a poor colloidal stability. The level of coagulable nitrogen in the finished wort is an important figure for the characterization of the efficiency of wort-boiling processes, and the evaluation and comparison of different boiling systems. The level of coagulable nitrogen in unboiled wort is in the range of 35–70 ppm and is reduced during boiling to 15–25 ppm with a recommended optimal value of 15–18 ppm. A low coagulable nitrogen concentration is beneficial for a good colloidal stability in the finished beer, but a too low concentration can result in head retention problems (Miedaner 1986). Protein coagulation is affected by physical and technological factors (Narziss 1992), as reviewed in Table 20.5.

Wort Sterilization and Enzyme Inactivation. Only a short boiling time is necessary to obtain a sterile solution. The microflora of the malt, hop, and other adjuncts are readily destroyed. The inactivation of residual enzymes, which survived the mashing process, is also necessary to fix the wort composition. There is only a residual activity of polyphenoloxidase and α-amylases in the wort before boiling, and a short boiling time is sufficient to denature these enzymes.

The Maillard Reaction. During wort boiling, the Maillard or nonenzymatic browning reaction is rather intensive, resulting in the production of various volatile and nonvolatile aroma compounds and colored melanoidins (brown nitrogenous polymers and

copolymers). The reaction starts with an interaction of low-MW proteins, that is, amino acids, and reducing sugars, and the Amadori rearrangement (Danehy 1986; Villamiel and others 2006). From there, a rather complex reaction network is described, including the Strecker degration. The progress of the Maillard reaction can be observed by an increase in wort color, by measuring the concentration of intermediate products (like 5-hydroxymethylfurfural (HMF), furfural, furfurylalcohol, 2-acetylfuran, 2-acetylpyrrol, and heterocyclic nitrogen compounds; Narziss and others 1983), or measuring the increase of the concentration of reductones using the "Indicator Time Test". A too intensive, uncontrolled reaction can lead to unattractive flavors in beers. The formed melanoidins are reducing compounds, but are also involved in the oxidation of higher alcohols in fresh beer, resulting in volatile aldehydes (Hashimoto 1972). Melanoidins can exert pro- and antioxidant effects (Ames 2001a). Although the structures of melanoidins isolated from foods are unknown (Ames and Nursten 1989; Ames 2001b), considerable progress has been made in recent years concerning the structures of melanoidin-like materials.

Formation of Coloring Substances. Wort boiling results in an increase in wort color: typically 4 EBC units for a light-colored beer. This increase is due to the formation of melanoidins, the caramelization of sugars and the oxidation of polyphenols. As the extent of the Maillard reaction is higher at a higher pH, the color increases with increasing pH of the wort. Thermal stress during wort boiling can also be monitored by the thiobarbituric acid coefficient (TBC), number (TBN), or index (TBI). The TBC values can also give (in combination with the coagulable nitrogen concentration) information about the expected foam stability of the produced beer (Wasmuht and Stippler 2000).

Removal of Unwanted Volatiles. During malting, *S*-methylmethionine (SMM) is formed. This compound is the precursor of dimethylsulfide (DMS), which gives a unpleasant smell and taste when present in the finished beer. At high temperatures, that is, during kilning, mashing (decoction method), and boiling, SMM is decomposed to DMS (Anness and Bamforth 1982). This DMS is very volatile and can be readily removed with the vapor during boiling. The transformation of SMM to DMS fits a first-order reaction with a half-lifetime of 30–70 min at 100°C (Narziss 1992). The formation of DMS is considerably lower at pH 5.0 compared to a pH of 5.5–5.8. This determines the lower pH limit value of the wort at the start of boiling. An optimal combination of boiling time and temperature has to be used, because when the boiling time is too low, the DMS concentration will be too high (but coagulable nitrogen can be acceptable); when the boiling time is too short, DMS concentration will also be too high (the coagulable nitrogen content can be correct) (Schwill-Miedaner and Miedaner 2001). On the other hand, a too high boiling temperature and too long boiling time will result in a too low coagulable nitrogen content.

The removal of other unwanted volatile compounds during boiling is also necessary. These volatiles can be classified into three groups: malt-derived volatiles, hop oils, and volatiles that are formed during wort boiling. Several unwanted volatiles have been detected in the vapor condensate during boiling; for example, a five-fold quantity of 2-acetylthiazole has to be evaporated (Wackerbauer 1983). Myrcene is a very volatile hop oil, which gives a harsh and unpleasant aroma. In contrast, β-caryophylene, β-farnesene, and humulene give a wanted hop oil aroma.

Acidification of the Wort. Upon boiling, the wort becomes slightly acidic (typically 0.1–0.3 pH units for a classical boiling process) due to the formation of melanoidins, the addition of hop acids, the precipitation of alkaline phosphates and the acidification

action of Ca^{2+} and Mg^{2+} ions with phosphates. The use of dark malts (intense Maillard reaction during kilning) will also give larger pH decrease compared to pale malts.

Evaporation of Water. Wort boiling results in the evaporation of water (and volatile organic components) and the concentration of the wort. During classical (conventional) atmospheric boiling 8–12% of the initial wort volume was evaporated (some breweries even boiled over 2 h with an evaporation rate of up to 18%). It has been shown that reduction of evaporation to as little as 2% can be achieved without hazard to flavor or other beer qualities such as bitterness, head retention, total nitrogen, and color (Buckee and Barrett 1982).

20.2.5.2 Energy Saving During Wort Boiling. The brewhouse is the biggest energy consumer in the brewery. The total electrical power requirements of the brewhouse accounts for 50% of the brewery requirements and 15% of the total heat energy requirements (Anon. 2001a). Since the oil crisis in 1972 and more recently since the introduction of stringent ecology laws and taxes, the reduction of the use of primary energy during wort boiling is of primary concern to the brewer. Today, various possibilities with regard to energy recuperation are available.

In the brewhouse, thermal energy is required to heat the mash and the wort, and to boil the wort. The energy required for mash and wort heating can be recovered during the wort cooling process. Cooling of the wort after boiling can be accomplished in two steps: before and after the whirlpool, as it has been shown in large-scale tests that cooling of the wort by casting out at a temperature of 89°C significantly reduces the TBC value (lower thermal stress) and improves the flavor stability of the produced beer (Coors and others 2000; Krottenthaler and Back 2001).

The recovered energy can be used for preheating and boiling of the wort, and for hot water production (Vollhalls 1994; Thüsing 2000). Energy recovery can be accomplished by reducing the boiling time, using a vapor condenser for the production of hot service water with or without wort heating, mechanical vapor compression, thermal vapor compression with or without wort heating (Fig. 20.4).

Thermal and mechanical vapor compression is mainly used in the atmospheric boiling method because the vapor compression demands calandria for larger amounts of wort. An outlet temperature of 107–108°C is required if a calandria in combination with low-pressure boiling is used. The choice of an energy recuperation system will depend on the total evaporation rate, the hot water demand and the costs for thermal and electrical energy (Thüsing 2000, 2001).

Energy Saving with Vapor Condenser. The evaporated water mass during boiling contains a high energy content. The condensation of 1 kg of steam into 1 kg of water at 100°C gives an energy of 2260 kJ. Considerable heat can be saved using a vapor condenser and an energy storage system (Fig. 20.5). Instead of a one-tank storage tank, a two-tank arrangement, one tank containing the hot water ($\pm$99°C), the other the used hot water ($\pm$80°C), of the energy storage system can also be used (Lösch and Körber 1984; Lenz 1994). Nowadays, a single-layer plate heat exchanger is used as the vapor condenser. The energy recovered by the condenser is stored in the energy saver and used to heat the lautered wort before boiling. The energy saver is a hot water displacement storage tank. The vapor condensate can be further cooled to approximately 30°C using cold water, which is normally a prerequisite for its discharge into a waste water system. The vapor condensate cooler can produce hot water at a temperature of ~85°C. Only 4–5% of the total evaporation is sufficient for the production of hot water exclusively for wort

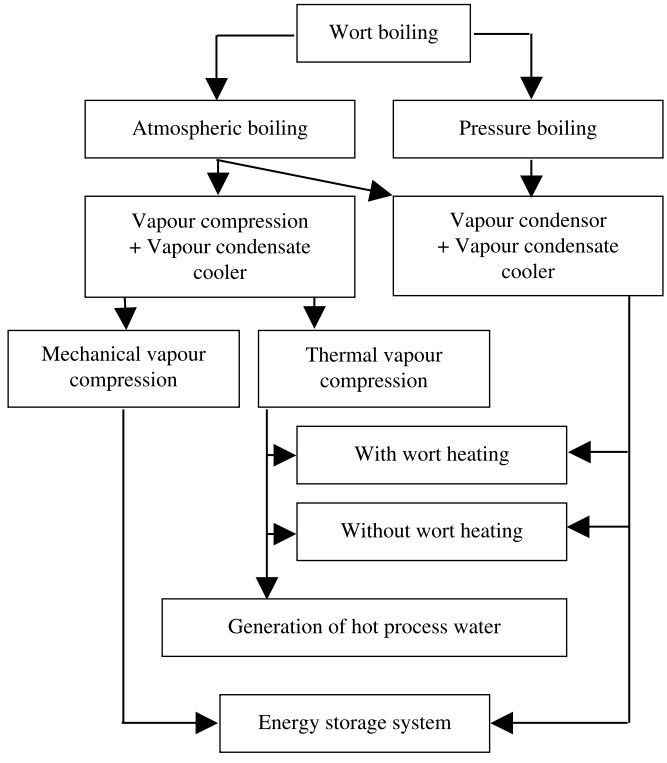

Figure 20.4 *Possibilities of energy recuperation with atmospheric or pressure boiling.*

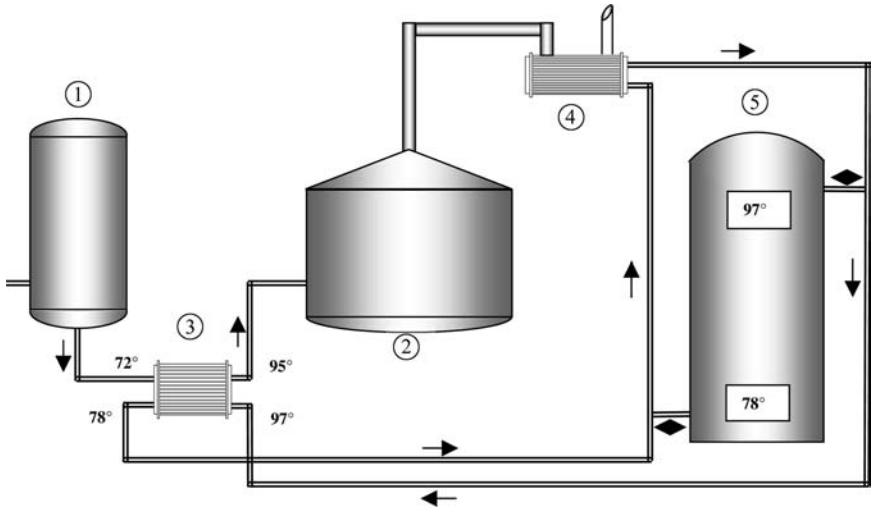

Figure 20.5 *Pressure wort copper with vapor condenser (pressureless); energy storage system and wort heating (after Vollhals, 1994); 1. wort collecting vessel, 2. wort kettle, 3. wort heater, 4. vapor condenser, 5. energy storage tank.*

heating. The same system can be used for low-pressure boiling. Low-pressure boiling works at a reduced evaporation rate compared to atmospheric boiling, which results in a lower production of hot service water. If the vapor condenser is included in the pressure sector, energy storage water at about 100°C can be produced.

Energy Saving with Vapor Compression. During atmospheric boiling, the produced vapor has a temperature of about 100°C. If this vapor is compressed to a few tenths of a bar overpressure, the temperature is raised to 102–108°C and it can be reused for heating. Vapor compression can be achieved by using a mechanical compressor or a steam jet compressor (thermocompression). The condensate, which is developed at the heater by the condensation of the vapor, leaves the cyclic process through a condensate cooler, which produces hot driving water.

Using a mechanical compressor, the vapor is compressed to an overpressure of 0.3–0.4 bar. The wort is heated to boiling temperature using fresh steam. The boiling process is maintained in operation using heat from the compressed vapor. The additional use of a vapor condenser is not possible because all the vapors are directly led back to the boiling process. Consequently, the lautered wort is not preheated when mechanical vapor compression is employed. Additional disadvantages are the complicated plant engineering, noise production, high maintenance costs, and peak electricity demands.

At thermal vapor compression, live steam from a boiler with an overpressure of at least 8 bar and up to 18 bar is fed to the steam jet pump. The vapor is sucked in and compressed to 0.1–0.4 bar overpressure. About 30–35% of the vapor is condensed in the kettle vapor condenser to produce hot water that can be used for preheating the wort. The advantages and disadvantages of using a thermal vapor compressor are summarized in Table 20.6.

20.2.5.3 Wort Boiling Systems. Today, different boiling systems are being used. Table 20.7 shows an overview. The new systems are characterized by a considerable reduced overall energy cost compared to classical systems. Investment costs depend on the system's complexity and ease of integration in the existing process. In Table 20.8, the various boiling systems are compared (classical atmospheric boiling with atmospheric venting is taken as the reference situation) with respect to thermal stress reduction,

TABLE 20.6 The Advantages and Disadvantages of Thermal Vapor Compression.

Advantages	Disadvantages
• A trouble-free, cheap, safe, and low-maintenance compressor • Low noise and vibration • Especially profitable for small and medium-sized breweries • Lower investment costs compared to mechanical vapor compression • Driven by live steam • One steam jet compressor is sufficient or any size of plant • Stepless control by injector needle valves	• Requires high steam pressure (new pipework needed) • A relative high quantity of hot process water arising • Large specific heat transfer surface with a high circulation rate (external heater needed or internal heater with recirculation pump) • A higher quantity of vapor condensate, waste, and hot water arising and more boiler feed water required compared to mechanical vapor compression

Source: Anon. (1993, 1995), Lambeck and Hintzen (1996), Fohr and Meyer-Pittroff (1998), Kunze (1999), Thüsing (2000).

TABLE 20.7 Overview of the Present-Day Boiling Systems.

Boiling System	Temperature (°C)	Boiling Time (min)	Total Evaporation Rate (%)
Classical atmospheric boiling	100	60–80	~8
Low pressure boiling (LPB)			
Classical	103–104[a]	55–65	6–7
Dynamic	103–104[a]	45–50	2.5–4
High temperature wort boiling (HTWB)	130–140	2.5–3	6–8
Low thermal load phase + stripping phase:			
Steam stripping: Meura system	100[b]	40–45	2.5–4
Film stripping: Merlin system	100[b]	35	5–6
Vacuum stripping:			
Ziemann system	100[b]	40–50	6
Nerb system	103[a]/99[b]	50–60	4.7–5.4
Schulz system	97.5[b]	60	8

Source: Willaert and Baron (2005).

[a]Boiler outlet.

[b]Temperature of the low thermal stress phase.

integration possibility in existing systems, obtained reduction in volatile organic compounds (VOCs) emission, achieved reduction of operation costs, and additional extra investment costs (Willaert and Baron 2005).

High-temperature boiling (HTWB) is a continuous boiling system, and the idea is quite old (Dumet 1958; Daris and others 1962; Evers 2002). At high temperatures of 130°C or 140°C, very satisfying wort analysis data can be obtained although very short boiling periods of ~5 min are used (Narziss and others 1982b; Narziss and others 1983). Narziss and others

TABLE 20.8 Comparison of the Different Boiling Systems.

Boiling System	Thermal Stress Reduction	Integration in Existing Systems	Reduction of VOC Emission[a]	Reduction of Operation Costs	Extra Investment Costs
Classical atmospheric boiling					
Atmospheric venting	−[b]	−	−	−	−
Vapor condensor	−	+++	+++	+	+
Vapor compression	−	−	+++	+	+++
Low-pressure boiling					
Classical boiling	+	−[c]	++/+++	++	+++[c]
Dynamic boiling	++	−[c]	++	++	+++[c]
High-temperature wort boiling	++	−	++/+++	+++	+++
Low thermal load phase + stripping phase:					
Steam stripping	+++	+++	+	+++	++
Film stripping	+++	+	++	+++	++
Vacuum stripping					
Ziemann system	+++	+++	++	+++	+++
Nerb system	+++	++	+/++	+++	+++
Kaspar Schultz	+++	+++	+++	+++	+++

Source: Willaert and Baron (2005).

[a]Based on the total evaporation rate.

[b]Classical atmospheric boiling with atmospheric venting is taken as the reference situation.

[c]Classical boiling vessels do not allow boiling under pressure; low-pressure boiling necessitates expensive pressure-resistant vessels.

(1991a,b) produced the best beers with a boiling temperature of 130°C and a high-temperature holding period of 180 s. The wort is heated up in three steps. In the first two steps, vapor from the flash-off chambers is reused. Considerable energy savings can be obtained due to the short boiling time and energy recovery. Alternative continuous systems have been developed, such as continuous pressure boiling in a multistage column comparable to a tray distillation tower (Krüger and Ehrlinger 1984) or the use of multiple-effect evaporators (Korek 1981).

A boiling system using microwaves was tested by the company Huppmann (Herrmann 1999; Isenberg 1999). Microwaves are produced in a separate generator. These microwaves are guided to the wort kettle via a copper waveguide and brought into the wort through the "applicator". The total wort volume was heated uniformly and no burn-on danger existed. Wort boiling trials (5 hL scale) with microwaves (4, 6, and 8% evaporation) have been compared to conventional boiling (90 min and 8% evaporation). The analysis of the obtained beers showed that the aroma was comparable to the beer obtained from the conventional boiling, although the boiling period could be reduced to 45 min (with 5% evaporation). The taste stability was rather poor for the produced "microwave beers" (Meilgaard 2001).

Wort Boiling at Low Pressure. Conventional boiling at low pressure was introduced in breweries at the end of the 1970s to decrease energy costs (Lenz 1982). The pressure is kept constant during boiling at a value of 1.08–1.21 bar (boiling temperature from 102 to 105°C). This technology has been further developed and the state of the art today is dynamic low-pressure boiling with several subsequent short phases of pressure building up and pressure release with corresponding multiple wort stripping (N.N. 2001b, Hackensellner 2001; Kantelberg and Hackensellner 2001; Schwill-Miedaner and Miedaner 2001). This technology has been commercialized by the company Huppmann (Kitzingen, Germany).

After an atmospheric preboiling phase of 3 min at 100°C, the pressure is periodically built up (1.17 bar, 104°C) and released (1.05 bar, 101°C). The boiling is ended with a post-boiling phase of 5 min at 100°C. The pressure reduction phases ensure an intensive boiling phase with stripping of wort volatiles. A total evaporation rate of 4.4–4.5% is obtained. The produced vapor is used to produce hot water and the recovered energy can be stored in a hot-water storage tank. Part of this stored energy can be used to heat up the wort before boiling. Recent results have shown that – due to the reduced thermal stress on the wort – the head retention of the produced beers increased (Anon. 2001c).

Heating up and boiling of the wort is performed using an internal boiler. Recent internal boiling systems have been designed to minimize the thermal load during heating and boiling (Hoefig 1994; Stippler and Wasmuht 1999a; Hackensellner 1999; Anon. 2001d; Kantelberg and Hackensellner 2001).

Wort Boiling in Combination with Stripping. In wort boiling, most reactions are only time/temperature dependent (Reed and Jordan 1991; Schwill-Miedaner 2002): inactivation of enzymes, the sterilization of the wort, extraction of hop compounds, isomerization of α-acids, coagulation of the protein fraction, lowering the pH, and formation of reducing and aromatic compounds. For all these reactions, no evaporation is needed. A simple hot wort stand at boiling temperature is enough to guarantee that these reactions occur. Additionally, this methodology ensures a low thermal load, which guarantees a balanced beer with good foam stability.

On the contrary, for a few specific goals evaporation is required, initially to achieve the desired gravity of the wort at the end of boiling. Secondly, the high evaporation rates generally applied are meant to eliminate unwanted volatile compounds. In classical wort boiling, evaporation ratios of 7–10% are common. Unfortunately, in conventional wort

boiling, large energy losses may occur, because a wort kettle is not necessarily designed for efficient stripping by evaporation. The effective evaporation surface per unit volume is very low, which renders the elimination of volatile compounds more difficult (Reed and Jordan 1991). For this reason a vigorous wort boil is generally required.

Recently, several new wort-boiling systems have been developed and introduced into breweries. Wort boiling and removal of unwanted volatiles (free DMS, degradation product of fat, Strecker aldehydes, and so on) are two separate processes. In this way, the thermal stress on the wort and heating costs can be reduced considerably. A wort clarification step before the stripping phase gives a better wort quality than the conventional reversed strategy where a very high thermal load was necessary during the boiling phase to avoid DMS formation during wort clarification. The removal of the unwanted volatiles can be performed at a much higher efficiency. Additionally, modern wort-boiling systems are equipped with efficient energy-recovery systems. The stripping of volatiles can be accomplished by steam, film, or vacuum stripping.

STEAM STRIPPING. Steam stripping is employed in a two-stage boiling system where volatile compounds are stripped in a "stripping column" (Baron and others 1997; Seldeslachts 1997; Bonacchelli and others 2001). In the first stage of the process, the wort is kept at wort boiling temperature in a wort kettle where no significant evaporation takes place (Fig. 20.6). The volatile compounds formed through chemical reactions and extracted from the added hops are accumulated in the wort. In the second stage, after wort clarification, the volatile compounds are eliminated, very efficiently, in the "stripping column", with steam.

FILM STRIPPING. The company Anton Steinecker Maschinenfabrik (Krones; Freising, Germany) have introduced a new boiling system, named "Merlin". The Merlin is a vessel containing a conical heating surface, which serves for boiling and evaporation (stripping) of the wort (Fig. 20.7) (Schu and Stolz 1999; Stippler and Wasmuht 1999b; Jacob and others 2001). A whirlpool positioned under the Merlin serves as a wort-holding vessel. A circulation pump is also necessary. For hop addition, the usual hop-dosing system is used. This system can also be coupled to an energy storage tank (Manger 2000; Weinzierl and others 2000; Schwill-Miedaner and Miedaner 2001).

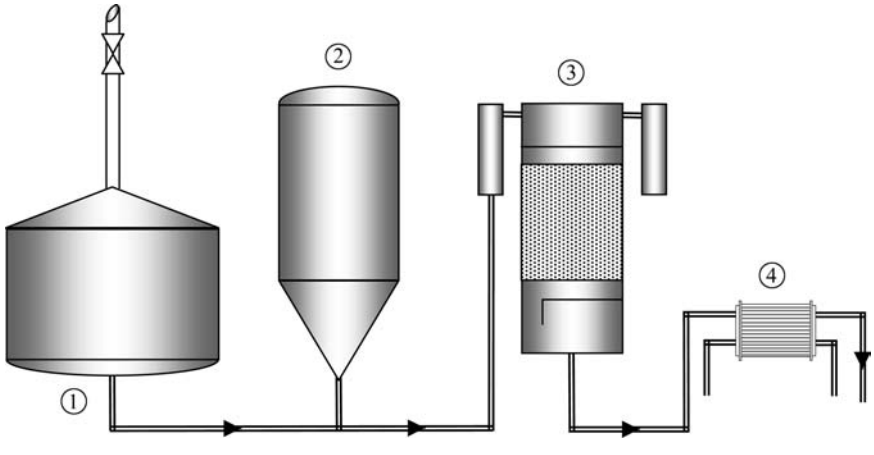

Figure 20.6 *Process layout including wort stripping (after Baron and others 1997); 1. wort kettle, 2. sedimentation tank (wort clarification), 3. wort stripper (packed with Cascade Rings from Glitsch), 4. wort cooler.*

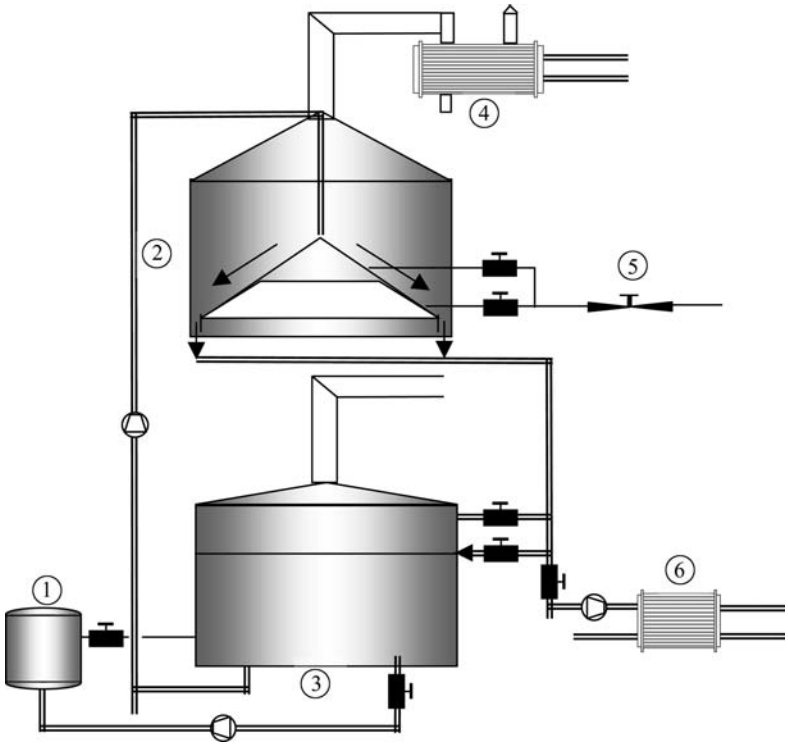

Figure 20.7 *Schematic of a brewhouse featuring Merlin and whirlpool with energy storage (after Schwill-Miedaner and Miedaner, 2001); 1. hop dosage, 2. Merlin, 3. whirlpool as collecting vessel, 4. vapor condenser, 5. steam control valve, 6. heat exchanger.*

The wort is heated until it reaches boiling temperature by pumping it in a circulation loop across the conical heating surface of the Merlin. When boiling temperature is reached in the whirlpool, a boiling cycle of 35 min starts. Next, there is a whirlpool phase of 20–30 min, followed by film stripping to remove unpleasant aroma volatiles. The stripping is accomplished by pumping the wort from the whirlpool once again across the heated surface of the Merlin on its way from whirlpool to plate cooler.

VACUUM STRIPPING

Ziemann System. The company Ziemann (Ludwigsburg, Germany) have introduced a vacuum evaporation plant, which can be installed in the brewhouse as an additional module (N.N. 2001b,e). This module is placed after the whirlpool. The combination of a relatively short boiling phase with a low evaporation rate and vacuum evaporation gives a boiling system with a low energy cost, a reduced thermal load, and sufficient unwanted volatile stripping.

The wort is boiled over 40–50 min with an existing boiling kettle (~4% evaporation). Next, the whirlpool is employed as usual. After the rest period in the whirlpool, the wort is led tangentially as a thin film through the bypass of the existing wort pipe into the

cylindroconical vacuum vessel. Undesired flavor compounds are removed during the flash evaporation process (~2% evaporation).

Nerb System. The company Nerb (Freising Attaching, Germany) introduced recently on the market the boiling system "VarioBoil" (Krottenthaler and others 2001; Anon. 2001b). This system combines atmospheric and vacuum boiling. This boiling system is composed of a wort vessel, an external boiler, an expansion evaporator with a vacuum pump, and a vapor condenser coupled to a hot-water storage tank. Additionally, vapor compression can also be used during boiling. Instead of the wort-boiling vessel, a buffer tank or whirlpool can be used.

The wort is pumped through the external boiler into the expansion vessel. The wort flows tangentially into this vessel and as a thin film from the inner surface. During boiling, the pressure is atmospheric in the expansion vessel. An outlet pump is used to pump the wort back to the wort kettle and to control the level in the expansion vessel. After the boiling process, the expansion vessel is evacuated. The wort is then pumped via the expansion vessel to the whirlpool.

Kaspar Schulz System. In the boiling system of the company Kaspar Schulz (Bamberg, Germany) (Anon. 2001f; Binkert and Haertl 2001), wort boiling is accomplished in two phases. In the first phase, the wort is kept at a temperature just below the boiling point (~97.5°C), and the second phase is a vacuum evaporation phase. A total evaporation rate of 8% is obtained. Considerable energy is saved by keeping the wort just below boiling point during the hot holding phase (because there is no evaporation energy cost).

The wort is heated in the wort kettle to 97.5°C and kept at this temperature for 60 min. The wort is stirred constantly with an impeller. The length of this hot holding period (~1% evaporation) is adjustable and depends on the isomerization yield of the α-acids and the required value of coagulable nitrogen. Next, the hot trub and hops debris are removed in a whirlpool. The clarified wort is pumped to the vacuum evaporator. An evaporation rate of 7% is obtained.

20.2.6 Wort Clarification

After boiling, it is necessary to remove the hot trub and hop debris to ensure flavor and colloidal stability of the beer and to avoid blocking of the plate heat exchanger during wort cooling. If hot trub particles are not removed, they will foul the yeast cell wall and also stimulate yeast sedimentation during fermentation. Consequently, a reduced fermentation degree will be obtained. Hot trub particles give beer a darker color, a rough trub taste, and a poor foam stability.

20.2.6.1 Spent Hops Removal. When hopping is performed with hop cones, the spent hops are removed before hot trub removal in a separate process step. Spent hops contain 5–6 L wort per kg hops. Therefore, spent hops are washed with hot water or washed and subsequently squeezed. Spent hops can be separated from the wort using the hop back or the hop strainer. In a hop back, the wort is pumped through a stirred vessel and spent hops are retained in the vessel using a sieve. For large wort volumes, a hop strainer is used. A screw conveyor just above the sieve squeezes the spent hops and "dry" spent hops are discarded.

20.2.6.2 Hot Trub Removal.

The hot trub ("coarse break", "coarse trub") particles have a size of 30–80 μm. It is composed, on average, of 40–70% proteinaceous material, 10–20% hop bitter acids, 7–8% polyphenols, 7–10% carbohydrates, 1–2% fatty acids, and varying quantities of minerals (Anon. 1999). The hot trub content of wort is typically 40–80 g/hL extract-free dry matter. This amount depends on the nitrogen content and cultivation conditions of barley, the type of mashing process, the mash filtration method, and boiling duration and intensity. The hot trub content of wort has to be decreased to less than 100 mg/L (Kunze 1999).

Different process techniques can be used to remove the hot trub from the wort. The separation processes are based on separating particles with a different density and/or size, and include sedimentation, centrifugation, and filtration techniques.

The easiest way to remove hot trub is by sedimentation by using a sedimentation tank or a whirlpool. A coolship has been used in the past as sedimentation vessel. It is a flat, rectangular, open vessel in which the wort is cast to a depth of 15–25 cm. Sedimentation takes about 0.5–2 h. This technology is still in use in Belgium for the production of lambic beers and related (fruit) beers (Gueuze, Kriek, Framboise). In this case, the wort is allowed to stand overnight until the required fermentation temperature is reached and to allow the wort to be inoculated spontaneously by the local microbiological flora, which is present in the circulating air.

The sedimentation tank is a circular vessel, usually designed with a conical bottom for easy removal of the trub. The tank is filled with hot wort and during a rest period the trub particles sediment and clarify the wort. A pivoted wort discharge pipe can be used to adjust the draw off point of the wort to just below the liquid surface. In some designs, wort can be discharged at different vessel heights. The wort–trub mixture can either be fed back to the lauter tun or can be treated separately (e.g., by centrifugation) to recover the entrained wort.

Hot trub removal in a whirlpool is one of the most elegant and economical methods. It is a cylindrical vessel into which wort is pumped tangentially. This produces a rotational flow in the vessel which causes the hot break to settle in the shape of a cone in the middle of the bottom of the vessel. Clarified wort is drawn off at the side.

Centrifugation is an effective technique to clarify the wort. The sedimentation speed is increased by increasing the sedimentation force (centrifugal vs. gravitational acceleration). The advantages of this method are that a centrifuge takes up a small space, it works continuously, emptying, cleaning, and sterilization are easy, and there are reduced wort losses (however, wort losses depend on the load of spent hop powder). The disadvantages of centrifugation are high requirements for electrical energy, noise, and relatively high maintenance costs.

Filtration is today the most used technique to remove cold trub. It can also be applied for hot trub removal. Small breweries use kieselguhr sheet filters ("plate and frame" filters). Larger breweries may use candle sieve filters that are adapted for wort clarification.

20.3 BEER FERMENTATION

20.3.1 Biochemistry of the Primary Beer Fermentation

20.3.1.1 Carbohydrate Metabolism: Ethanol Production.

Carbohydrate Uptake. Carbohydrates in wort make up 90–92% of wort solids. Wort from barley malt contains the fermentable sugars sucrose, fructose, glucose, maltose,

TABLE 20.9 Carbohydrate Composition of Worts.

Origin Type of Wort Original Gravity	Danish Lager 1043.0	Canadian Lager 1054.0	British Pale Ale 1040.0
Fructose (g/L)	2.1	1.5	3.3
(%)[a]	2.7	1.6	4.8
Glucose (g/L)	9.1	10.3	10.0
(%)[a]	11.6	10.9	14.5
Sucrose (g/L)	2.3	4.2	5.3
(%)[a]	2.9	4.5	7.7
Maltose (g/L)	52.4	60.4	38.9
(%)[a]	66.6	64.2	56.5
Maltotriose (g/L)	12.8	17.7	11.4
(%)[a]	16.3	18.8	16.5
Total ferm. Sugars (g/L)	78.7	94.1	68.9
Maltotetraose (g/L)	2.6	7.2	2.0
Higher sugars (g/L)	21.3	26.8	25.2
Total dextrins (g/L)	23.9	34.0	25.2
Total sugars (g/L)	102.6	128.1	94.1

Source: Adapted from Hough and others (1982).
[a]Percent of the total fermentable sugars.

and maltotriose, together with some dextrin material (Table 20.9). The fermentable sugars typically make up 70–80% of the total carbohydrate (MacWilliam 1968). The three major fermentable sugars are glucose, and the α-glucosides maltose and maltotriose. Maltose is by far the most abundant of these sugars, typically accounting for 50–70% of the total fermentable sugars in an all-malt wort. Sucrose and fructose are present in a low concentration. The unfermentable dextrins play little part in brewing. Wort fermentability may be reduced or increased by using solid or liquid adjuncts.

Brewing strains consume the wort sugars in a specific sequence: glucose is consumed first, followed by fructose, maltose, and finally maltotriose. The uptake and consumption of maltose and maltotriose is repressed or inactivated at elevated glucose concentrations. Only when 60% of the wort glucose has been taken up by the yeast will uptake and consumption of maltose start. Maltotriose uptake is inhibited by high glucose and maltose concentrations. When high amounts of carbohydrate adjuncts (e.g., glucose) or high-gravity wort are employed, the glucose repression is even more pronounced, resulting in fermentation delays (Stewart and Russell 1993).

The efficiency of brewer's yeast strains to effect alcoholic fermentation is dependent upon their ability to utilize the sugars present in wort. This ability very largely determines the fermentation rate as well as the final quality of the beer produced. In order to optimize the fermentation efficiency of the primary fermentation, a detailed knowledge of the sugar consumption kinetics, which is linked to the yeast growth kinetics, is required (Willaert 2001).

MALTOSE AND MALTOTRIOSE METABOLISM. The yeast *S. cerevisiae* transports the monosaccharides across the cell membrane using hexose transporters. There are 19, or possibly 20, genes encoding hexose transporters (Dickinson 1999). The disaccharide maltose and the trisaccharide maltotriose are transported by specific transporters into the cytoplasm, where these molecules are hydrolyzed by the same α-glucosidase, yielding two or three molecules of glucose, respectively (Panchal and Stewart 1979; Zheng and others 1994).

Maltose utilization in yeast is conferred by any one of five *MAL* loci: *MAL1* to *MAL4* and *MAL6* (Bisson and others 1993; Dickinson 1999). Each locus consists of three genes: gene 1 encodes a maltose transporter (permease), gene 2 encodes a maltase (α-glucosidase), and gene 3 encodes a transcriptional activator of the other two genes. Thus, for example, the maltose transporter gene at the *MAL1* locus is designated *MAL61*. The three genes of a *MAL* locus are all required to allow fermentation. Some authors persist in using gene designations such as for the *MAL1* locus: *MAL1T* (transporter = permease), *MAL1R* (regulator), and *MAL1S* (maltase). The five *MAL* loci each map to a different chromosome. The *MAL* loci exhibit a very high degree of homology and are telomere linked, suggesting that they evolved by translocation from telomeric regions of different chromosomes (Michels and others 1992). Because a fully functional or partial allele of the *MAL1* locus is found in all strains of *S. cerevisiae*, this locus is proposed as the progenitor of the other *MAL* loci (Chow and others 1983). Gene dosage studies performed with laboratory strains of yeast have shown that the transport of maltose in the cell may be the rate-limiting step in the utilization of this sugar (Goldenthal and others 1987). Constitutive expression of the maltose transporter gene (*MALT*) with high-copy-number plasmids in a lager strain of yeast has been found to accelerate the fermentation of maltose during high-gravity (24°P) brewing (Kodama and others 1995). The constitutive expression of *MALS* and *MALR* had no effect on maltose fermentability. Recently, it was confirmed that maltose uptake is the dominant factor controlling the rate of maltose utilization in wort (11–24°P) fermentations using an ale and lager industrial strain (Rautio and Londesborough 2003).

The control over *MAL* gene expression is exerted at three levels. The presence of maltose induces, whereas glucose represses, the transcription of *MALS* and *MALT* genes (Federoff and others 1983a,b; Needleman and others 1984). The constitutively expressed regulatory protein (*MALR*) binds near the *MALS* and *MALT* promotors and mediates the induction of *MALS* and *MALT* transcription (Cohen and others 1984; Chang and others 1988; Ni and Needleman 1990). Experiments with *MALR*-disrupted strains led to the conclusion that MalRp is involved in glucose repression (Goldenthal and Vanoni 1990; Yao and others 1994). Relatively little attention has been paid to post-transcriptional control, that is, the control of translational efficiency, or mRNA turnover, as mechanisms complementing glucose repression (Soler and others 1987). The addition of glucose to induced cells has been reported to cause a 70% increase in the lability of a mRNA population containing a fragment of *MALS* (Federoff and others 1983a). The third level of control is post-translational modification. In the presence of glucose, maltose permease is either reversibly converted to a conformational variant with decreased affinity (Siro and Lövgren 1979; Peinado and Loureiro-Dias 1986) or irreversibly proteolytically degraded depending on the physiological conditions (Lucero and others 1993; Riballo and others 1995). The latter phenomenon is called catabolite inactivation. Glucose repression is accomplished by the Mig1p repressor protein which is encoded by the *MIG1* gene (Nehlin and Ronne 1990). It has been shown that Mig1p represses the transcription of all three *MAL* genes by binding upstream of them (Hu and others 1995). The *MIG1* gene has been disrupted in a haploid laboratory strain and in an industrial polyploid strain of *S. cerevisiae* (Klein and others 1996). In the *MIG1*-disrupted haploid strain, glucose repression was partly alleviated; that is, maltose metabolism was initiated at higher glucose concentrations than in the corresponding wild-type strain. In contrast, the polyploid *Δmig1* strain exhibited an even more stringent glucose control of maltose metabolism than the corresponding wild-type strain, which could be explained by a more rigid catabolite inactivation of maltose permease, affecting the uptake of maltose.

Recently, the gene *AGT1*, which codes for an α-glucoside transporter, has been characterized (Han and others 1995). *AGT1* is found in many *S. cerevisiae* laboratory strains and maps to a naturally occurring, partially functional allele of the *MAL1* locus. Agt1p is a highly hydrophobic, postulated integral membrane protein. It is 57% identical to Mal61p (the maltose permease encoded at *MAL6*) and is also a member of the 12 transmembrane domain superfamily of sugar transporters (Nelissen and others 1995). Like Mal61p, Agt1p is a high-affinity, maltose/proton symporter, but Mal61p is capable of transporting only maltose and turanose, and Agt1p transports these two α-glucosides as well as several others including isomaltose, α-methylglucoside, maltotriose, palatinose, trehalose, and melezitose. *AGT1* expression is maltose inducible and induction is mediated by the Mal-activator.

Brewing strains of yeast are polyploid, aneuploid, or, in the case of lager strains, alloploid. Recently, Jespersen and others (1999) examined 30 brewing strains of yeast (5 ale strains and 25 lager strains), with the aim of examining the alleles of maltose and maltotriose transporter genes contained by them. All the strains of brewer's yeast examined, except two, were found to contain *MAL11* and *MAL31* sequences, and only one of these strains lacked *MAL41*. *MAL21* was not present in the five ale strains and 12 of the lager strains. *MAL61* was not found in any of the yeast chromosomes other than those known to carry *MAL* loci. Sequences corresponding to the *AGT1* gene (transport of maltose and maltotriose) were detected in all but one of the yeast strains.

The utilization of maltose or sucrose by a selection of 25 brewing, baking, or laboratory strains of *S. cerevisiae* was either repressible, constitutive or absent (Meneses and Jiranek 2002a). Overall fermentation rate of a selection of nine strains showed a good correlation with maximum specific maltose transport rate, but poor correlation with maximum maltase activity, implying that transport rather than hydrolysis of maltose was the rate-limiting step (Meneses and Jiranek 2002b). All strains were found to possess genomic sequences detectable with probes for the *MAL11*, *MAL31*, and *SUC2* invertase gene. However, the loci present and the pattern of expression of these and other *MAL* genes varied widely. From comparison of sugar utilization and gene expression patterns, constitutive maltose utilization in strain NCYC 1681 was best explained by *MAL11* expression. However, such expression of *MAL11* was not mirrored by changes in the expression of the transcriptional activator(s) (*MALx3*).

Wort maltotriose has the lowest priority for uptake by brewer's yeast cells and incomplete maltotriose uptake results in yeast fermentable extract in beer, material loss, greater potential for microbiological stability, and sometimes atypical beer flavor profiles (Stewart and Russell 1993). Maltotriose uptake from wort is always slower with ale strains than with lager strains under similar fermentation conditions. However, the initial transport rates are similar to those of maltose in a number of ale and lager strains. Elevated osmotic pressure inhibits the transport and uptake of glucose, maltose and maltotriose with maltose and maltotriose being more sensitive to osmotic pressure than glucose in both lager and ale strains. Ethanol (5% w/v) stimulated the transport of maltose and maltotriose, due in all probability to an ethanol-induced change in the plasma membrane configuration, but had no effect on glucose transport. Higher ethanol concentrations inhibited the transport of all three sugars.

Wort Fermentation. Before the fermentation process starts, wort is aerated (see Section 20.3.3.1). As a result of this aeration step, carbohydrates are degraded aerobically during

the first few hours of the fermentation process. The aerobic carbohydrate catabolism typically takes 12 h for a lager fermentation.

During the first hours of the fermentation process, oxidative degradation of carbohydrates occurs through the glycolysis and Krebs (TCA) cycle. The energy efficiency of glucose oxidation is derived from the large number of $NADH_2^+$ produced for each mole of glucose oxidized to CO_2. The actual wort fermentation produces, alcohol and carbon dioxide *via* the Embden–Meyerhof–Parnas (glycolytic) pathway. The reductive pathway from pyruvate to ethanol is important because it regenerates NAD^+. Energy is obtained solely from ATP-producing steps of the Embden–Meyerhof–Parnas pathway. During fermentation, the activity of the TCA cycle is greatly reduced, although it still serves as a source of intermediates for biosynthesis (Lievense and Lim 1982).

Lagunas (1979) observed that during aerobic growth of *S. cerevisiae*, respiration accounts for less than 10% of glucose catabolism, the remainder being fermented. Increasing sugar concentrations resulting in a decreased oxidative metabolism is known as the Crabtree effect. This was traditionally explained as an inhibition of the oxidative system by high concentrations of glucose. Nowadays, it is generally accepted that the formation of ethanol at aerobic conditions is a consequence of a bottleneck in the oxidation of pyruvate, for example, in the respiratory system (Petrik and others 1983; Rieger and others 1983; Käppeli and others 1985; Fraleigh and others 1989; Alexander and Jeffries 1990).

A reduction of ethanol production can be achieved by metabolic engineering of the carbon flux in yeast resulting in an increased formation of other fermentation products. A shift of the carbon flux towards glycerol at the expense of ethanol formation in yeast was achieved by simply increasing the level of glycerol-3-phosphate dehydrogenase (Michnick and others 1997; Nevoigt and Stahl 1997; Remize and others 1999; Dequin 2001). The *GDP1* gene, which encodes glycerol-3-phosphate dehydrogenase, has been overexpressed in an industrial lager brewing yeast to reduce the ethanol content in beer (Nevoigt and others 2002). The amount of glycerol produced by the *GDP1*-overexpressing yeast in fermentation experiments – simulating brewing conditions – was increased 5.6 times and ethanol was decreased by 18% compared to the wild-type strain. Overexpression did not affect the consumption of wort sugars and only minor changes in the concentration of higher alcohols, esters, and fatty acids could be observed. However, the concentrations of several other byproducts, particularly acetoin, diacetyl, and acetaldehyde, were considerably increased.

20.3.1.2 Metabolism of Bioflavoring Byproducts.

Yeast is an important contributor to flavor development in fermented beverages. The compounds, which are produced during fermentation, are many and varied, depending on both the raw materials and the yeast strains used. The interrelation between yeast metabolism and the production of bioflavoring byproducts is illustrated in Figure 20.8.

Biosynthesis of Higher Alcohols. During beer fermentation, higher alcohols (also called "fusel alcohols") are produced by yeast cells as byproducts and represent the major fraction of the volatile compounds. More than 35 higher alcohols in beer have been described. Table 20.10 gives the most important compounds, which can be classified into aliphatic (n-propanol, isobutanol, 2-methylbutanol (or active amyl alcohol), and 3-methylbutanol (or isoamyl alcohol)) and aromatic (2-phenylethanol, tyrosol, tryptophol) higher alcohols. Aliphatic higher alcohols contribute to the "alcoholic" or "solvent" aroma of beer, and produce a warm mouthfeel. The aromatic alcohol 2-phenylethanol has a sweet aroma and

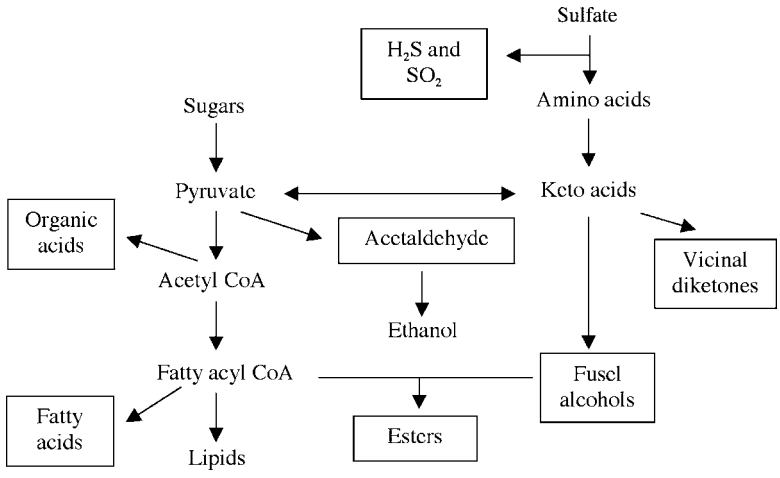

Figure 20.8 *Interrelationships between yeast metabolism and the production of flavor active compounds (Hammond 1993).*

has a positive contribution to the beer aroma, whereas the aroma of tyrosol and tryptophol are undesirable.

Higher alcohols are synthesized by yeast during fermentation via the catabolic (Ehrlich) and anabolic pathway (amino acid metabolism) (Ehrlich 1904; Chen 1978; Oshita and others 1995; Dickinson 1999). In the catabolic pathway, the yeast uses the amino acids of the wort to produce the corresponding α-keto acid via a transamination reaction. The excess oxoacids are subsequently decarboxylated into aldehydes and further reduced (alcohol dehydrogenase) to higher alcohols. This last reduction step also regenerates NAD^+.

Dickinson and co-workers looked at the genes and enzymes, that are used by *S. cerevisiae* in the catabolism of leucine to isoamyl alcohol (Dickinson and others 1997), valine to isobutanol (Dickinson and others 1998), and isoleucine to active amyl alcohol (Dickinson and others 2000). In all cases, the general sequence of biochemical reactions is similar, but the details for the formation of the individual alcohols are

TABLE 20.10 Major Higher Alcohols in Beer.

Compound	Flavor Threshold (mg/L)	Aroma or taste[b]	Concentration Range (mg/L) Bottom Fermentation	Concentration Range (mg/L) Top Fermentation
n-Propanol	600[c], 800[b]	Alcohol	7–19 (12)*[f]	20–45[i]
Isobutanol	100[c], 80–100[g], 200[b]	Alcohol	4–20 (12)[f]	10–24[i]
2-Methylbutanol	50[c], 50–60[g], 70[b]	Alcohol	9–25 (15)[a]	80–140[i]
3-Methylbutanol	50[c], 50–60[g], 65[b]	Fusely, pungent	25–75 (46)[a]	80–140[i]
2-Phenylethanol	5[a], 40[c], 45–50[g], 75[d], 125[b]	Roses, sweetish	11–51 (28)[f], 4–22[g], 16–42[h]	35–50[g], 8–25[a], 18–45[i]
Tyrosol	10[a], 10–20[e], 20[c], 100[d,g], 200[b]	Bitter chemical	6–9[a], 6–15[a]	8–12[g], 7–22[g]
Tryptophol	10[a], 10–20[e], 200[d]	Almonds, solvent	0.5–14[a]	2–12[g]

Source: Anon. (2000).

*Mean value.

[a]Szlavko (1973), [b]Meilgaard (1975a), [c]Engan (1972), [d]Rosculet (1971), [e]Charalambous and others (1972); [f]Values in 48 European lagers, Dufour (unpublished data), [g]Reed and Nogodawithana (1991), [h]Iverson (1994), [i]Derdelinckx (unpublished data).

surprisingly different. The branched-chain amino acids are first deaminated to the corresponding α-ketoacids (α-ketoisocapric acid from leucine, α-ketoisovaleric acid from valine and α-keto-β-methylvaleric acid from leucine). There are significant differences in the way each α-ketoacid is subsequently decarboxylated. Recently, the catabolism of phenylalanine to 2-phenylethanol and of tryptophan were also studied (Dickinson and others 2003). Phenylalanine and tryptophan are first deaminated to 3-phenylpyruvate and 3-indolepyruvate, respectively, and then decarboxylated. These studies revealed that all amino acid catabolic pathways studied to date use a subtly different spectrum of decarboxylases from the five-membered family that comprises Pdc1p, Pdc5p, Pdc6p, Ydl080cp, and Ydr380wp. Using strains containing all possible combinations of mutations affecting the seven *AAD* genes (putative aryl alcohol dehydrogenases), five *ADH* and *SFA1* (other alcohol dehydrogenase) genes, showed that the final step of amino acid catabolism can be accomplished by any one of the ethanol dehydrogenases (Ahd1p, Ahd2p, Ahd3p, Ahd4p, Ahd5p) or Sfa1p (formaldehyde dehydrogenase).

In the anabolic pathway, the higher alcohols are synthesized from α-keto acids during the synthesis of amino acids from the carbohydrate source (Oshita and others 1995). The pathway choice depends on the individual higher alcohol and on the level of available amino acids available. The importance of the anabolic pathway decreases as the number of carbon atoms in the alcohol increases (Chen 1978), and increases in the later stage of fermentation as wort amino acids are depleted (MacDonald and others 1984). Yeast strain, fermentation conditions, and wort composition all have significant effects on the combination and levels of the higher alcohols that are formed (MacDonald and others 1984; Boulton and Quain 2001).

Conditions that promote yeast cell growth – such as high levels of nutrients (amino acids, oxygen, lipids, zinc ...), increased temperature and agitation – stimulate the production of higher alcohols (Landaud and others 2001). The synthesis of aromatic alcohols is especially sensitive to temperature changes. On the other hand, conditions that restrict yeast growth – such as lower temperature and higher pressure – reduce the extent of higher alcohol production (Renger and others 1992; Landaud and others 2001). Amino acid composition has a major effect on higher alcohol formation. Growth medium supplemented with valine, isoleucine and leucine induced the formation of isobutanol, amyl alcohol, and isoamyl alcohol, respectively (Äyräpää 1971; Sablayrolles and Ball 1995; Kodama and others 2001). Constitutive expression of the *BAP2* gene, which codes for the branched-chain amino acid permease Bap2p, resulted in accelerated rates of assimilation for leucine, valine, and isoleucine (Kodama and others 2001). This caused an increased production of isoamyl alcohol derived from leucine, and an increase of isobutyl alcohol derived from valine or active amyl alcohol from isoleucine was not observed. These results suggest that there are distinct but interrelated mechanisms for the production of each higher alcohol.

Biosynthesis of Esters. Esters are very important flavor compounds in beer. They have an effect on the fruity/flowery aromas (Verstrepen and others 2003a). Table 20.11 shows the most important esters with their threshold values, which are considerably lower than those for higher alcohols. The major esters can be subdivided into acetate esters and C_6–C_{10} medium-chain fatty acid ethyl esters. They are desirable components of beer when present in appropriate quantities and proportions, but can become unpleasant when in excess. Ester formation is highly dependent on the yeast strain used (Nykänen and Nykänen 1977; Peddie 1990) and on certain fermentation parameters such as temperature (Engan and Aubert 1977; Gee and Ramirez 1994; Sablayrolles and Ball 1995), specific growth rate (Gee and Ramirez 1994), pitching rate (Maule 1967; D'Amore and others 1991; Gee and

TABLE 20.11 Major Esters in Beer.

Compound	Flavor Threshold (mg/L)	Aroma	Concentration Range (mg/L) in 48 Lagers
Ethyl acetate	20–30, 30[a]	Fruity, solvent-like	8–32 (18.4)*
Isoamyl acetate	0.6–1.2, 1.2[a]	Banana, peardrop	0.3–3.8 (1.72)*
Ethyl caproate (ethyl hexanoate)	0.17–0.21, 0.21[a]	Apple–like with note of aniseed	0.05–0.3 (0.14)*
Ethyl caprylate (ethyl octanoate)	0.3–0.9, 0.9[a]	Apple-like	0.04–0.53 (0.17)*
2-Phenylethyl acetate	3.8[a]	Roses, honey, apple, sweetish	0.10–0.73 (0.54)*

Source: Dufour and Malcorps (1994).
*Mean value.
[a]Meilgaard (1975b).

Ramirez 1994) and top pressure (Anon. 2000). Additionally, the concentrations of assimilable nitrogen compounds (Hammond 1993; Calderbank and Hammond 1994; Sablayrolles and Ball 1995), carbon sources (Pfisterer and Stewart 1975; White and Portno 1979; Younis and Stewart 1998, 2000), dissolved oxygen (Anderson and Kirsop 1975a,b; Avhenainen and Mäkinen 1989; Sablayrolles and Ball 1995) and fatty acids (Thurston and others 1981, 1982) can influence the ester production rate. Fermentation of high-gravity worts results in a disproportionate synthesis of esters, particularly ethyl acetate and isoamylacetate (Anderson and Kirsop 1974, 1975a, 1975b; Casey and others 1985; Stewart 2001).

Esters are produced by yeast both during the growth phase (60%) and also during the stationary phase (40%) (Anon. 2000). They are formed by the intracellular reaction between a fatty acyl-coenzyme A and an alcohol:

$$R'OH + RCO{-}ScoA \longrightarrow RCOOR' + CoASH. \quad (1)$$

This reaction is catalyzed by an alcohol acyltransferase (or ester synthethase). Because acetyl CoA is also a central molecule in the synthesis of lipids and sterols, ester synthesis is linked to the fatty acid metabolism (see also Fig. 20.8).

Alcohol acetyltransferase (AAT) has been localized in the plasma membrane (Malcorps and Dufour 1987) and found to be strongly inhibited by unsaturated fatty acids, ergosterol, heavy metal ions, and sulfydryl reagents (Minetoki and others 1993). Subcellular fractionation studies conducted during the batch fermentation cycle demonstrated the existence of both cytosolic and membrane-bound AAT (Ramos-Jeunehomme and others 1989, 1991). In terms of controlling ester formation on a metabolic basis, it has further been shown that ester-synthesizing activity of AAT is dependent on its positioning within the yeast cell. An interesting feature of this distribution pattern is that specific rates of acetate ester formation varied directly with the level of cytosolic AAT activity (Masschelein 1997).

The *ATF1* gene, which encodes alcohol acetyltransferase, has been cloned from *S. cerevisiae* and brewery lager yeast (*S. cerevisiae uvarum*) (Fujii and others 1994). A hydrophobicity analysis suggested that alcohol acetyltransferase does not have a membrane-spanning region that is significantly hydrophobic, which contradicts the membrane-bound assumption. A Southern analysis of the yeast genomes in which the *ATF1* gene was used as a probe, revealed that *S. cerevisiae* has one *ATF1* gene, and

brewery lager yeast has one *ATF1* gene and another, homologous gene (*Lg-ATF1*). The AAT activities have been compared *in vivo* and *in vitro* under different fermentation conditions (Malcorps and others 1991). This study suggested that ester synthesis is modulated by a repression–induction of enzyme synthesis or processing, the regulation of which is presumably linked to lipid metabolism.

Ester production can be altered by changing the synthesis rate of certain fusel alcohols. Hirata and others (1992) increased the isoamyl acetate levels by introducing extra copies of the *LEU4* gene in the *S. cerevisiae* genome. A comparable *S. cerevisiae uvarum* mutant has been isolated (Lee and others 1995). The mutants have an altered regulation pattern of amino acid metabolism and produce more isoamyl acetate and phenylethyl acetate.

Isoamyl acetate is synthesized from isoamyl alcohol and acetyl coenzyme A by AAT and is hydrolyzed by esterases at the same time in *S. cerevisiae*. To study the effect of balancing both enzyme activities, yeast strains with different numbers of copies of the *ATF1* gene, and isoamyl acetate-hydrolyzing esterase gene (*IAH1*) have been constructed and used in small-scale sake brewing (Fukuda and others 1998). Fermentation profiles as well as components of the resulting sake were largely alike. However, the amount of isoamyl acetate in the sake increased with increasing ratio of AAT/Iah1p esterase activity. Therefore, it was concluded that the balance of these two enzyme activities is important for isoamyl acetate accumulation in sake mash.

The synthesis of acetate esters by *S. cerevisiae* during fermentation is ascribed to at least three acetyltransferase activities, namely alcohol acetyltransferase (AAT), ethanol acetyltransferase, and isoamyl AAT (Lilly and others 2000). To investigate the effect of increased AAT activity on the sensory quality of Chenin blanc wines and distillates from Colombar based wines, the *ATF1* gene of *S. cerevisiae* was overexpressed. Northern blot analysis indicated constitutive expression of *ATF1* at high levels in these transformants. The levels of ethyl acetate, isoamyl acetate, and 2-phenylethyl acetate increased 3- to 10-fold, 3.8- to 12-fold, and 2- to 10-fold, respectively, depending on the fermentation temperature, cultivar, and yeast used. The concentrations of ethyl caprate, ethyl caprylate, and hexyl acetate only showed minor changes, whereas the acetic acid concentration decreased by more than half. This study established the concept that the overexpression of acetyltransferase genes such as *ATF1* could profoundly affect the flavor profiles of wines and distillates deficient in aroma.

In order to investigate and compare the roles of the known *S. cerevisiae* alcohol acetyltransferases, Atf1p, Atf2p, and Lg-Atf1p, in volatile ester production, the respective genes were either deleted or overexpressed in a laboratory strain and a commercial brewing strain (Verstrepen and others 2003b). Analysis of the fermentation products confirmed that the expression levels of *ATF1* and *ATF2* greatly affect the production of ethyl acetate and isoamyl acetate. GC-MS analysis revealed that Atf1p and Atf2p are also responsible for the formation of a broad range of less volatile esters, such as propyl acetate, isobutyl acetate, pentyl acetate, hexyl acetate, heptyl acetate, octyl acetate, and phenyl ethyl acetate. With respect to the esters analyzed in this study, Atf2p seemed to play only a minor role compared to Atf1p. The *atf1 atf2* double deletion strain did not form any isoamyl acetate, showing that together, Atf1p and Atf2p are responsible for the total cellular isoamyl alcohol acetyltransferase activity. However, the double deletion strain still produced considerable amounts of certain other esters, such as ethyl acetate (50% of the wild-type strain), propyl acetate (50%), and isobutyl acetate (40%), which provides evidence for the existence of additional, as-yet-unknown ester synthases in the yeast proteome. Interestingly, overexpression of different alleles of *ATF1* and *ATF2* led

to different ester production rates, indicating that differences in the aroma profiles of yeast strains may be partially due to mutations in their *ATF* genes.

Recently, it has been discovered that the Atf1 enzyme is localized inside lipid vesicles in the cytoplasm of the yeast cell (Verstrepen 2003). Lipid vesicles are small organelles in which certain neutral lipids are metabolized or stored. This indicates that fruity esters are possibly byproducts of these processes.

Biosynthesis of Organic Acids. Important organic acids detected in beer include acetate, lactate, succinate, pyroglutamate, malate, citrate, α-ketoglutarate and α-hydroxyglutarate (Coote and Kirsop 1974). They influence flavor directly when present above their taste threshold, and by their influence on beer pH. These components have their origin in raw materials (malt, hops) and are produced during beer fermentation. Organic acids that are excreted by yeast cells, are synthesized via amino acid biosynthesis pathways and carbohydrate metabolism. In particular, they are overflow products of the incomplete Krebs cycle during beer fermentation. Excretion of organic acids is influenced by yeast strain and fermentation vigor. Sluggish fermentations lead to lower levels of excretion. Pyruvate excretion follows the yeast growth: maximal concentration is reached just before the maximal yeast growth, and is next taken up by the yeast and converted to acetate. Acetate is synthesized quickly during early fermentation and is later partially reused by the yeast during yeast growth. At the end of the fermentation, acetate is accumulated. The reduction of pyruvate results in the production of D-lactate or L-lactate (most yeast strains produce preferentially D-lactate). The highest amount of lactate is produced during the most active fermentation period.

The change in organic acid productivity by disruption of the gene encoding fumarase (*FUM1*) has been investigated and it has been suggested that malate and succinate are produced *via* the oxidative pathway of the TCA cycle under static and sake brewing conditions (Magarifuchi and others 1995). Using a NAD^+-dependent isocitrate dehydrogenase gene (*IDH1*, *IDH2*) disruptant, approximately half of the succinate in sake mash was found to be synthesized *via* the oxidative pathway of the TCA cycle in sake yeast (Asano and others 1999).

Sake yeast strains possessing various organic acid productivities were isolated by gene disruption (Arikawa and others 1999). Sake fermented using the aconitase gene (*ACO1*) disruptant contained a two-fold higher concentration of malate and a two-fold lower concentration of succinate than that made using the wild-type strain. The fumarate reductase gene (*OSM1*) disruptant produced sake containing a 1.5-fold higher concentration of succinate, whereas the α-ketoglutarate dehydrogenase gene (*KGD1*) and fumarase gene (*FUM1*) disruptants gave lower succinate concentrations. In *S. cerevisiae*, there are two isoenzymes of fumarate reductase (FRDS1 and FDRS2), encoded by the *FRDS* and *OSM1* genes, respectively (Arikawa and others 1998). Recent results suggest that these isoenzymes are required for the reoxidation of intracellular NADH under anaerobic conditions, but not under aerobic conditions (Enomoto and others 2002).

Succinate dehydrogenase is an enzyme of the TCA cycle and thus essential for respiration. In *S. cerevisiae*, this enzyme is composed of four nonidentical subunits, that is, the flavoprotein, the iron-sulfur protein, the cytochrome b_{560}, and the ubiquinone reduction protein encoded by the *SDH1*, *SDH2*, *SDH3*, and *SDH4* genes, respectively (Lombardo and others 1990; Chapman and others 1992; Bullis and Lamire 1994; Daignan-Fournier and others 1994). Sdh1p and Sdh2p comprise the catalytic domain involved in succinate oxidation. These proteins are anchored to the inner mitochondrial membrane by

Sdh3p and Sdh4p, which are necessary for electron transfer and ubiquinone reduction, and constitute the succinate:ubiquinone oxidoreductase (complex II) of the electron transport chain. Single or double disruptants of the *SDH1*, *SDH1b* (which is a homolog of the *SDH1* gene), *SDH2*, *SDH3*, and *SDH4* genes have been constructed and shown that the succinate dehydrogenase activity was retained in the *SDH2* disruptant and that double disruption of *SDH1* and *SDH2* or *SDH1b* genes is necessary to cause deficiency of succinate dehydrogenase activity in sake yeast (Kubo and others 2000). The role of each subunit in succinate dehydrogenase activity and the effect of succinate dehydrogenase on succinate production using strains that were deficient in succinate dehydrogenase, have also been determined. The results suggested that succinate dehydrogenase activity contributes to succinate production under shaking conditions, but not under static and sake brewing conditions.

Biosynthesis of Vicinal Diketones. Vicinal diketones are ketones with two adjacent carbonyl groups. During fermentations, these flavor-active compounds are produced as byproducts of the synthesis pathways of isoleucine, leucine, and valine (ILV pathway) (Fig. 20.9), and thus are also linked to amino acid metabolism (Nakatani and others 1984) and the synthesis of higher alcohols. They impart a "buttery", "butterscotch" aroma to alcoholic drinks. Two of these compounds are important in beer: diacetyl (2,3-butanedione) and 2,3-pentanedione. Diacetyl is quantitatively more important than 2,3-pentanedione. It has a taste threshold at around 0.10–0.15 mg/L in lager beer, approximately 10 times lower than that of pentanedione (Wainwright 1973).

The excreted α-acetohydroxy acids are overflow products of the ILV pathway that are nonenzymatically degraded to the corresponding vicinal diketones (Inoue and others 1968). Tetraploid gene dosage series for various *ILV* genes have been constructed and the obtained yeast strains were used to study the influence of the copy number of *ILV* genes on the production of vicinal diketones (Debourg and others 1990; Debourg 2002). It was shown that *ILV5* activity is the rate-limiting step in the ILV pathway and responsible for the overflow (Fig. 20.9). The nonenzymatic oxidative decarboxylation step is the rate limiting step and proceeds faster at a higher temperature and a lower pH (Inoue and Yamamoto 1970; Haukeli and Lie 1978). The produced amount of α-acetolactate is very dependent on the yeast strain used. The production increases with

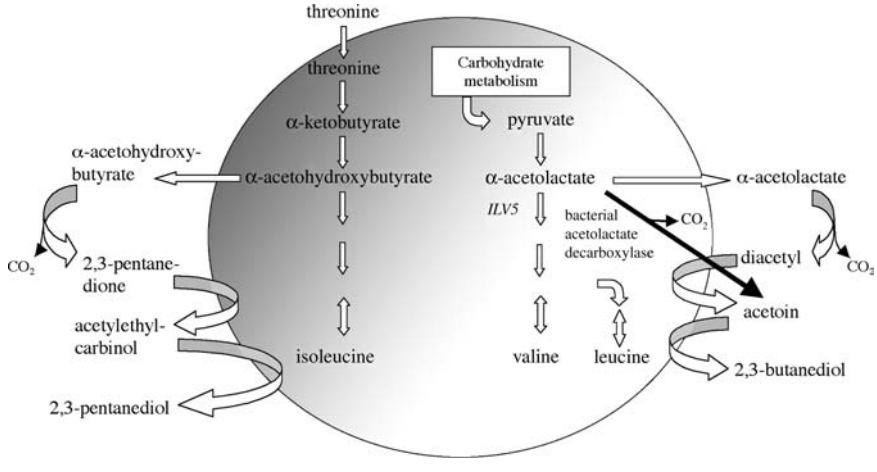

Figure 20.9 *The synthesis and reduction of vicinal diketones in S. cerevisiae.*

increasing yeast growth. For a classical fermentation, 0.6 ppm α-acetolactate is formed (Delvaux 1998). At high aeration, this value can be increased to 0.9 ppm and in cylindroconical fermentation tanks even to 1.2–1.5 ppm.

Yeast cells posses the necessary enzymes (alcohol dehydrogenase, diacetyl reductases) to reduce diacetyl to acetoin and further to 2,3-butanediol, and 2,3-pentanedione to 2,3-pentanediol (Hardwick and others 1976; Louis-Eugene and others 1988; Legeay and others 1989; Heidlas and Tressl 1990; Bamforth and Kanauchi 2004). These reduced compounds have much higher taste thresholds and have no impact on beer flavor (Van Den Berg and others 1983). The reduction reactions are yeast-strain-dependent. The reduction occurs at the end of the main fermentation and during the maturation. Sufficient yeast cells in suspension are necessary to obtain an efficient reduction. Yeast strains that flocculate early during the main fermentation need a long maturation time to reduce the vicinal diketones. Diacetyl can be complexed using SO_2. These complexes cannot be reduced, but diacetyl can again be liberated at a later stage by aldehydes. This situation is especially applicable to yeast strains that produce a lot of SO_2. Worts that are produced using much adjuncts can be low in free amino acid content. These worts can give rise to a high diacetyl peak at the end of the fermentation.

There are several strategies that can be chosen to reduce the amount of vicinal diketones during fermentation:

1. As the temperature has a positive effect on the reduction efficiency of the α-acetohydroxy acids, a warm rest period at the end of the main fermentation and a warm maturation are applied in many breweries. In this case, temperature should be well controlled to avoid yeast autolysis.
2. As the rapid removal of vicinal diketones requires yeast cells in an active metabolic condition, the addition of 5–10% Krausen (containing active, growing yeast) is a procedure that gives enhanced transformation of vicinal diketones (N.N. 2000). This procedure can lead to overproduction of hydrogen sulfide, depending upon the proportions of threonine and methionine carried forward from primary fermentation.
3. Heating up green beer to a high temperature (90°C) and holding it there for a short period (~7–10 min) to decarboxylate all excreted α-acetohydroxy acids. To avoid cell autolysis, yeast cells are removed by centrifugation prior to heating up. The vicinal diketones can be further reduced by immobilized yeast cells in a few hours (typically at 4°C) (see p. 488, Flavor maturation of green beer).
4. Adding the enzyme α-acetolactate decarboxylase (Godtfredsen and others 1984; Rostgaard-Jensen and others 1987; Hannemann 2002). This enzyme decarboxylates α-acetolactate directly into acetoin (Fig. 20.9). It is not present in *S. cerevisiae*, but has been isolated from various bacteria such as *Enterobacter aerogenes*, *Aerobacter aerogenes*, *Streptococcos lactis*, *Lactobacillus casei*, *Acetobacter aceti*, and *Acetobacter pasteurianus*. It has been shown that the addition of α-acetolactate decarboxylase from *Lactobacillus casei* can reduce the maturation time to 22 h (Godtfredsen and others 1983, 1984). An example of a commercial product is Maturex L from Novo Nordisk (Denmark) (Jensen 1993). Maturex L is a purified α-acetolactate decarboxylase produced by a genetically modified strain of *Bacillus subtilis*, which has received the gene from *Bacillus brevis*. The recommended dosage is 1–2 kg per 1000 hL wort, to be added to the cold wort at the beginning of fermentation.

5. Using genetic modified yeast strains.
   a. Introducing the bacterial α-acetolactate decarboxylase gene into yeast chromosomes (Fujii and others 1990; Suihko and others 1990; Blomqvist and others 1991; Enari and others 1992; Linko and others 1993; Yamano and others 1994; Tada and others 1995; Onnela and others 1996). Transformants possessed a very high α-acetolactate decarboxylase activity, which reduces the diacetyl concentration considerably during beer fermentation.
   b. Modifying the biosynthetic flux through the ILV pathway. Spontaneous mutants resistant to the herbicide sulfometuron methyl have been selected. These strains showed a partial inactivation of the α-acetolactate synthase activity and some mutants produced 50% less diacetyl compared to the parental strain (Gjermansen and others 1988).
   c. Increasing the flux of α-acetolactate acid isomeroreductace activity encoded by the *ILV5* gene (Dillemans and others 1987). Because α-acetolactate acid isomeroreductace activity is responsible for the rate-limiting step, increasing its activity reduces the overflow of α-acetolactate. A multicopy transformant results in a 70% decreased production of vicinal diketones (Villaneuba and others 1990), whereas an integrative transformant gives a 50% reduction (Goossens and others 1993). A tandem integration of multiple *ILV5* copies results also in elevated transciption in a polyploidy industrial yeast strain (Mithieux and Weiss 1995). Plasmid amplification of the gene products of *ILV5*, *ILV3*, or tandem *ILV5* + *ILV3* results in vicinal diketone decreases of 70, 40, and 60%, respectively, when compared to that of normal brewer's yeast (Villa and others 1995).

20.3.2 Biochemistry of Secondary Beer Fermentation

During the secondary fermentation or maturation of beer several objectives should be realized:

- Sedimentation of yeast cells;
- Improvement of colloidal stability by sedimentation of the tannin–protein complexes;
- Beer saturation with carbon dioxide;
- Removal of unwanted aroma compounds;
- Excretion of flavor-active compounds from yeast to give body and depth to the beer;
- Fermentation of the remaining extract;
- Improvement of the foam stability of the beer;
- Adjustment of the beer color (if necessary) by adding coloring substances (e.g., caramel);
- Adjustment of the bitterness of beer (if necessary) by adding hop products.

In the presence of yeast, the principal changes that occur are the elimination of undesirable flavor compounds – like vicinal diketones, hydrogen sulfide, and acetaldehyde – and the excretion of compounds enhancing the palate fullness (body) of beer.

20.3.2.1 Vicinal Diketones. In traditional fermentation lagering processes, the elimination of vicinal diketones required several weeks and determined the length of

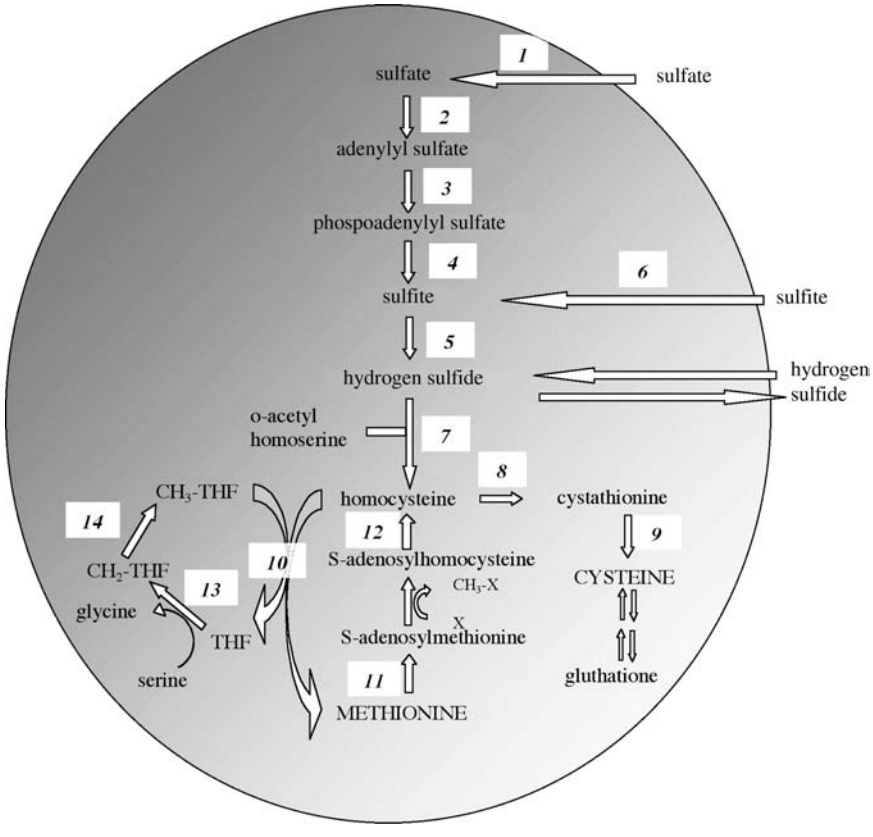

Figure 20.10 *The re-methylation, transulfuration, and sulfur assimilation pathways. Genes and enzymes catalyzing individual reactions are: 1. sulfate permease, 2. ATP sulfurylase, 3. MET14: adenylylsulfate kinase (EC 2.7.1.25), 5. MET10: sulfite reductase (EC 1.8.1.2), 6. sulfite permease, 7. MET17: O-acetylhomoserine (thiol)-lyase (EC 2.5.1.49), 8. CYS4: cystathionine β-synthase (CBS; EC 4.2.1.22), 9. CYS3: cystathionine γ-lyase (EC 4.4.1.1), 10. MET6: methionine synthase (EC 2.1.1.14), 11. SAM1 and SAM2: S-adenosylmethionine synthetase (EC 2.5.1.6), 12. SAH1: S-adenosylhomocysteine hydrolase (EC 3.3.1.1), 13. SHM1 and SHM2: serine hydroxymethyltransferase (SHMT; EC 2.1.2.1), 14. MET12 and MET13: methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20). "X" represents any methyl group acceptor; THF, tetrahydrofolate; CH_2-THF, 5,10-methylenetetrahydrofolate; CH_3-THF, 5-methyltetrahydrofolate (partly adapted from Chan and Appling 2003).*

the maturation process. Nowadays, the maturation phase is much shorter because strategies are used to accelerate the removal of vicinal diketones (see above). Diacetyl is used as a marker molecule. The objective during lagering is to reduce the diacetyl concentration below its taste threshold (<0.10 mg/mL).

20.3.2.2 Hydrogen Sulfide. Hydrogen sulfide plays an important role during maturation. Hydrogen sulfide that is not incorporated into S-containing amino acids is excreted by the yeast cell during the growth phase (Fig. 20.10). The excreted amount depends on the used yeast strain, the sulfate content of the wort and the growth conditions (Romano and Suzzi 1992). At the end of the primary fermentation and during the maturation, the excess H_2S is reutilized by the yeast. A warm conditioning period at $10-12^{\circ}$C may be used to remove excessive levels of H_2S.

Brewing yeasts produce H_2S when they are deficient in the vitamin pantothenate (Walker 1998). This vitamin is a precursor of coenzyme A, which is required for metabolism of sulfate into methionine. Panthothenate deficiency may therefore result in an imbalance in sulfur–amino acid biosynthesis, leading to excess sulfate uptake and excretion of H_2S (Slaughter and Jordan 1986).

Sulfite is a versatile food additive used to preserve a large range of beverages and foodstuffs. In beer, sulfite has a dual purpose, acting both as an antioxidant and an agent for masking of certain off-flavors. Some of the flavor-stabilizing properties of sulfite are suggested to be due to complex formation of bisulfate with varying carbonyl compounds, of which some would give rise to off-flavors in bottled beer (Dufour 1991). In particular, the unwanted carbonyl *trans*-2-nonenal has received particular attention, because it is responsible for the "cardboard" flavor of some types of stale beer. It has been suggested that it would be better to use a yeast strain with reduced sulfite excretion during fermentation and to add sulfite at the point of bottling to ensure a good flavor stability (Francke Johannesen and others 1999). Therefore, a brewer's yeast disabled in the production of sulfite has been constructed by inactivating both copies of the two alleles of the *MET14* gene (which encodes for adenylylsulfate kinase). Fermentation experiments showed that there was no qualitative difference between yeast-derived and artificially added sulfite, with respect to *trans*-2-nonenal content and flavor stability of the final beer.

The elimination of the gene encoding sulfite reductase (*MET10*) in brewing strains of *Saccharomyces* results in increased accumulation of SO_2 in beer (Hansen and Kielbrandt 1996a). The inactivation of *MET2* results in elevated sulfite concentrations in beer (Hansen and Kielbrandt 1996b). Beers produced with increased levels of sulfite have shown an improved flavor stability.

The production of H_2S could be reduced by the expression of cystathione synthase genes from *S. cerevisiae* in a brewing yeast strain (Tezuka and others 1992).

20.3.2.3 Acetaldehyde. Aldehydes – in particular acetaldehyde (green apple-like flavor) – have an impact on the flavor of green beer. Acetaldehyde synthesis is linked to yeast growth. Its concentration is maximal at the end of the growth phase, and is reduced at the end of the primary fermentation and during maturation by the yeast cells. As with diacetyl, levels may be enhanced if yeast metabolism is stimulated during transfer, especially by oxygen ingress. Removal also requires the presence of enough active yeast. Fermentations with early flocculating yeast cells can result in too high acetaldehyde concentrations at the end.

20.3.2.4 Development of Palate Fullness. During maturation, the residual yeast will excrete compounds (i.e., amino acids, phosphates, peptides, nucleic acids, . . .) into the beer. The amount and quality of these excreted materials depend on the yeast concentration, yeast strain, its metabolic state, and the temperature (N.N. 2000). Rapid excretion of material is best achieved at a temperature of 5–7°C over 10 days (Van de Meersche and others 1977).

When the conditioning period is too long or when the temperature is too high, yeast cell autolysis will occur. Some enzymes are liberated (e.g., α-glucosidase), which will produce glucose from traces of residual maltose (N.N. 2000). At the bottom of a fermentation tank, the amount of α-amino-nitrogen can rise to 40–10,000 mg/L, which accounts for an increase of 30 mg/L for the total beer volume. The increase in amino acid concentration in the beer has a positive effect on the flavor fullness of the beer. Undesirable medium-chain fatty acids can also be produced in significant amounts if the maturation temperature is too

high (Masschelein 1981). Measurement of these compounds indicates the level of autolysis and permits the determination of the most appropriate conditioning period and temperature.

20.3.3 Beer Fermentation Technology

20.3.3.1 Batch Fermentation Technology. In modern breweries, the primary (main) as well as the secondary fermentation (maturation) are performed in cylindroconical tanks (CCTs). The concept of using CCTs for beer fermentation is quite old. Leopold Nathan patented CCT systems for beer fermentation in 1908 and 1927. It is only since the 1960s, however, that they have been used extensively (Maule 1986). These vessels have a number of desirable features (N.N. 2000):

- They occupy a small land area in relation to volume;
- They can be used for all beer types;
- An increased dispersal of yeast by carbon dioxide uplift allows rapid fermentation;
- Ease of pressure application, carbon dioxide collection, yeast and cold break removal and cleaning (using a CIP system);
- Efficient cooling; and
- Simplified automation.

The content of CCTs can range from 1000 to 10,000 hL. Vessels with high height-to-diameter ratios lead to increased hydrostatic pressure, which leads to overcarbonation. In vessels with a very high height-to-diameter ratio, amino acid utilization by the yeast is enhanced due to the increased fluid flow circulation via carbon dioxide evolution. This results in a beer with increased higher alcohol and decreased ester content. CCTs are made from nonrusting chrome–nickel (V2A) steel. The temperature is closely regulated using temperature sensors and cooling jackets (or cooling pockets) at different heights to remove the heat that is produced during fermentation. Direct or indirect cooling techniques can be used. In direct cooling, liquid ammonia is evaporated (which withdraws heat from the fermenting wort) in the cooling jacket. In indirect cooling, glycol is usually used as the cooling medium, which is cooled in a cooling plant using a plate heat exchanger.

To obtain a rapid start of fermentation, the wort needs to be aerated because yeast cells contain insufficient levels of sterols and unsaturated fatty acids, which need to be incorporated into the yeast cell membrane (David and Kirsop 1973; Rogers and Stewart 1973), and the synthesis of these compounds requires dissolved oxygen (O'Connor-Cox and others 1993). It has been shown that ergosterol and unsaturated fatty acids both increase in concentration as long as oxygen is present in the wort (Haukeli and Lie 1979). A maximum concentration is obtained 5–6 h after pitching, but the formation rate is dependent upon pitching rate and temperature. An interesting new alternative process is to preoxygenate the yeast cells before fermentation. Studies on lager (Aries and Kirsop 1978; Ohno and Takahashi 1986; Devuyst and others 1991; Masschelein and others 1995) and ale (Depraetere and others 2003) yeast preoxygenation have shown that sterols and unsaturated fatty acids are synthesized upon yeast preoxygenation, which results in an improved physiological condition of the yeast cells. The build-up of sterols and unsaturated fatty acids varies considerably among different lager and yeast strains (Depraetere and others 2003). Therefore, a specific preoxygenation procedure for every yeast strain is necessary to obtain successful results. Recent studies have revealed that the supplementation of

cropped yeast with the unsaturated fatty acid, linoleic acid, results in increased ratios of unsaturated fatty acids to total fatty acids and trehalose to glycogen (Moonjai and others 2000, 2002), indicating that supplementation of unsaturated fatty acids is also an interesting alternative to wort aeration.

Different devices are used to aerate the cold wort: ceramic or sintered metal candles, aeration plants employing venturi pipes, two-component jets, static mixers, or centrifugal mixers (Kunze 1999). The principle of these devices is that very small air (oxygen) bubbles are produced and quickly dissolve during turbulent mixing.

A consistent pitching (yeast addition) rate is important for uniform fermentations. Generally, brewers pitch from $10-15 \times 10^6$ viable cells/mL (Knudsen 1985; Narziss 2005) to $15-20 \times 10^6$ cells/mL (Anon. 2000) into regular 12°P wort, up to 25×10^6 cells/mL for 18°P high-gravity worts, and as low as 6×10^6 cells/mL for 9°P low-gravity wort (Knudsen 1985). A rule of thumb for many is to use one million cells per degree Plato of the wort. The yeast to be used for pitching must be checked for viability. The viability must be kept at its highest possible level (95%) for constant performance.

Using CCTs, primary fermentation and maturation can be performed in the same vessel (single-vessel processing). A typical single-vessel procedure for the production of lager beer is summarized in Table 20.12.

At the end of the primary fermentation, yeast flocculation should occur consistently. Yeast flocculation is a reversable, asexual, and calcium-dependent process in which cells adhere to form flocs consisting of thousands of cells (Stratford 1989; Bony and others 1997; Jin and Speers 1999). Many fungi contain a family of cell-wall glycoproteins (called "adhesines") that confer unique adhesion properties (Teunissen and Steensma

TABLE 20.12 Typical Single-Vessel Procedure for the Production of Lager Beer.

Time Phase	Action/Feature	Temperature	Pressure	Density
Start	Pitching with $15-20 \times 10^6$ cells/mL	7–8°C	Atmospheric	12°P
24 h	Remove trub	Increased gradually to 8–11°C	Atmospheric	
End of growth phase	Improved conversion of acetolactate to diacetyl	Increased to 12–14°C and maintained until desired diacetyl level is attained	Allowed to rise to 0.3–0.5 bar overpressure	7–5°P
Just before end of main fermentation	Cooling of the conical bottom	5°C	Increasing overpressure	
24 h after conical bottom cooling	Initial yeast removal		Increasing overpressure	
Reaching of the desired diacetyl level (start maturation)	Slow cooling of the beer	Cooled to 5–7°C	2.5–3 bar overpressure	2–2.5°P
End maturation	Second yeast removal	5–7°C	2.5–3 bar overpressure	2–2.5°P
Colloidal stabilization	Maintained for 2–3 days	−1 to −2°C	2.5–3 bar overpressure	2–2.5°P
End colloidal stabilization	Final yeast removal	−1 to −2°C	2.5–3 bar overpressure	2–2.5°P

Source: Adapted from Anon. (2000).

1995; Guo and others 2000; Hoyer 2001). These molecules are required for the interactions of fungal cells with each other (flocculation and filamentation) (Teunissen and Steensma 1995; Lo and Dranginis 1998; Guo and others 2000; Viyas and others 2003), with inert surfaces such as agar and plastic (Gaur and Klotz 1997; Lo and Dranginis 1998; Reynolds and Fink 2001), and with mammalian tissues (Cormack and others 1999; Li and Palecek 2003); they are also crucial for the formation of fungal biofilms (Baillie and Douglas 1999; Reynolds and Fink 2001; Green and others 2004). The adhesin protein family in *S. cerevisiae* is responsible for its flocculation. The adhesin proteins are encoded by genes including *FLO1*, *FLO5*, *FLO9*, and *FLO10* (Verstrepen and others 2004). These proteins are called flocculins (Caro and others 1997), because they promote cell–cell adhesion to form multicellular clumps that sediment out of solution. The *FLO1*, *FLO5*, *FLO9*, and *FLO10* genes share considerable sequence homology. The member proteins of the adhesin family have a modular configuration that consists of three domains (A, B, and C) and an amino-terminal secretory sequence that must be removed as the protein moves through the secretory pathway to the plasma membrane (Hoyer and others 1998). The N-terminal domain (A) is involved in sugar recognition (Kobayashi and others 1998). The adhesins undergo several post-translational modifications. They move from the endoplasmic reticulum (ER), through the Golgi, and pass through the plasma membrane and find their final destination in the cell wall, where they are anchored by a glycosyl phosphatidylinositol (GPI) (Teunissen and others 1993; Bidard and others 1994; Hoyer and others 1998; Bony and others 1997). The GPI anchor is added to the C terminus in the ER and mannose residues are added to the many serine and threonine residues in domain B in the Golgi (Udenfriend and Kodukula 1995; Bony and others 1997; Frieman and Cormack 2003; De Groot and others 2003). The *FLO1* gene product (Flo1p) has been localized at the cell surface by immunofluorescent microscopy (Bidard and others 1995). The amount of Flo proteins in flocculent strains increases during batch yeast growth and Flo1p availability at the cell surface determines the flocculation degree of the yeast. Flo proteins are polarly incorporated into the cell wall at the bud tip and the mother – daughter neck junction (Bony and others 1997). The transcriptional activity of the flocculation genes is influenced by the nutritional status of the yeast cells as well as other stress factors (Verstrepen and others 2003). This implies that during beer fermentation, flocculation is affected by numerous parameters such as nutrient conditions, dissolved oxygen, pH, fermentation temperature, and yeast handling and storage conditions.

20.3.3.2 Beer Fermentation Using Immobilized Cell Technology. The advantages of continuous fermentation – such as greater efficiency in utilization of carbohydrates and better use of equipment – led also to the development of continuous beer fermentation processes. Since the beginning of the twentieth century, many different systems using suspended yeast cells have been developed. The excitement for continuous beer fermentation led – especially during the 1950 and 1960s – to the development of various interesting systems. These systems can be classified as:

1. Stirred versus unstirred tank reactors;
2. Single-vessel systems versus a number of vessels connected in series; and
3. Vessels that allow yeast to overflow freely with the beer ("open system") versus vessels that have abnormally high yeast concentrations ("closed" or "semiclosed" systems) (Hough and others 1982; Wellhoener 1954; Coutts 1957; Bishop 1970).

However, these continuous beer fermentation processes were not commercially successful because of many practical problems, such as the increased danger of contamination (not only during fermentation but also during storage of wort in supplementary holdings tanks, which are required because the upstream and downstream brewing processes are usually not continuous), changes in beer flavor (Thorne 1968) and a poor understanding of the beer fermentation kinetics under continuous conditions. One of the well-known exceptions is the successful implementation of a continuous beer production process in New Zealand by Morton Coutts (Dominion Breweries), which is still in use today (Hough and others 1982; Coutts 1957).

In the 1970s, there was a revival in developing continuous beer fermentation systems due to the progress in research on immobilization bioprocesses using living cells. Narziss and Hellich (1971) developed one of the first well-described ICT processes for beer production. Yeast cells were immobilized in kieselguhr (which is widely used in the brewing industry as a filter aid), and a kieselguhr filter was employed as the bioreactor (called the "bio-brew bioreactor"). This process was characterized by a very low residence time of 2.5 h, but required the addition of viable yeast and a 7-day maturation period to reduce the high concentration of vicinal diketones in the green beer. Although this result looks very good, the bio-brew bioreactor, overall, gave no satisfying results. The most serious problem was the high amount of α-acetolactate in the green beer (Narziss 1997).

Baker and Kirsop (1973) were the first to use heat treatment of green beer to considerably accelerate the chemical conversion of α-acetolactate to diacetyl. They designed a two-step continuous process. The first reactor is a packed-bed reactor also containing kieselguhr with immobilized yeast cells to perform the primary fermentation. The green beer was heated using a heating coil to accelerate the α-acetolactate conversion. It was next cooled before it entered a smaller packed-bed reactor to perform the secondary fermentation. Problems associated with this process included a gradual blocking of the packed bed and a changed beer flavor.

In 1973, the first industrial-scale process using gel (polyacrylamide, later substituted by κ-carrageenan) immobilized nonliving *Escherichia coli* cells (containing aspartase activity) to produce L-aspartic acid from fumaric acid was implemented by the company Tanebe Seiyaku in Japan (Shibatani 1996). Following the introduction of alginate gel as an entrapment matrix for living yeast cells for the production of beer by White and Portno in 1979, there was an increased level of research activity in the development of ICT processes for the production of beer. In a laboratory-scale tower fermentor, these researchers were able to produce beer with a flavor that was comparable to the control (batch fermentation with free cells). This process was operational over 7 months. It was found that gel entrapment gave protection against contaminating bacteria. Furthermore, the concentration of ethyl acetate decreased during long-term operation.

Over the last 30 years, immobilized cell technology (ICT) has been extensively examined and some designs have already reached commercial exploitation. One of the major advantage of ICT is the increased volumetric productivity due to the high volumetric cell densities of yeast in the reactor. Consequently, the fermentation process can be performed in a smaller bioreactor (decreased capital costs) with shorter residence times. Immobilized cell systems are heterogeneous systems in which considerable mass transfer limitations can occur, resulting in a changed cell yeast metabolism. Therefore, successful exploitation of ICT needs a thorough understanding of mass transfer and intrinsic yeast kinetic behavior of these systems. Technical challenges include removal of excess yeast and carbon dioxide, sustaining yeast viability, optimization of oxygen (air) feed, prevention of

microbial contamination, prevention of clogging or channeling in some bioreactor types, and regeneration of large amounts of carrier material (Linko and others 1998).

Carrier Materials. Various cell immobilization carrier materials have been tested and used for beer production/bioflavoring. Selection criteria are summarized in Table 20.13. Depending on the particular application, reactor type, and operational conditions, some selection criteria will be more appropriate. Examples of selected carrier materials for particular applications are described in Table 20.14.

In one of the first ICT processes for continuous beer fermentation, kieselguhr (diatomaceous earth) was selected as the carrier material (Narziss and Hellich 1971). Later, alginate hydrogel encapsulation became popular (White and Portno 1979; Hsu and Bernstein 1985; Onaka and others 1985; Curin and others 1987; Shindo and others 1994; Nedovic and others 1997, 2001, 2004). In addition, some new hydrogel materials were introduced, such as κ-carrageenan, pectate gels, and polyvinyl alcohol (PVA) (Mensour and others 1996; Pilkington and others 1999; Smogrovicová and Dömény 1999; Raymond and others 2004; Nedovic and others 2005). The main advantage of the hydrogel entrapment method is the attainment of extremely high cell loadings and – consequently – high fermentation rates. However, in some cases cell proliferation and activity can be limited by low mass transfer rates within the matrices. The reduced cell growth in immobilized conditions can result in an insufficient free amino nitrogen consumption and, as a consequence, an unbalanced flavor profile of the final beer (Curin and others 1987; Hayes and others 1991). This was particularly the case for immobilized cells in packed-bed

TABLE 20.13 Selection Criteria for Yeast Cell Immobilization Carrier Materials.

- High cell mass loading capacity
- Easy access to nutrient media
- Simple and gentle immobilization procedure
- Immobilization compounds approved for food applications
- High surface-area-to-volume ratio
- Optimum mass transfer distance from flowing media to center of support
- Mechanical stability (compression, abrasion)
- Chemical stability
- Highly flexible: rapid start-up after shut-down
- Sterilizable and reusable
- Suitable for conventional reactor systems
- Low shear experienced by cells
- Easy separation of cells and carrier from media
- Readily up-scalable
- Economically feasible (low capital and operating costs)
- Desired flavor profile and consistent product
- Complete attenuation
- Controlled oxygenation
- Control of contamination
- Controlled yeast growth
- Wide choice of yeast

Source: Nedovic and Willaert (2004).

TABLE 20.14 Some Selected Applications of Cell Immobilization Systems Used for Beer Production.

Carrier Material	Reactor Type	Reference
	Flavor Maturation	
Calcium alginate beads	Fixed-bed	Shindo and others 1994
DEAE–cellulose	Fixed-bed	Pajunen and Grönqvist 1994
Polyvinyl alcohol beads	Fixed-bed	Smogrovicová and others 2001
Porous glass beads	Fixed-bed	Linko and others 1993, Aivasidis 1996
	Alcohol-Free Beer	
DEAE–cellulose beads	Fixed-bed	Collin and others 1991, Lomni and others 1990
Porous glass beads	Fixed-bed	Aivasidis and others 1991
Silicon carbide rods	Monolith reactor	Van de Winkel and others 1991
	Acidified Wort	
DEAE–cellulose beads	Fixed-bed	Pittner and others 1993
	Main Fermentation	
Calcium alginate beads	Gas-lift	Nedovic and others 1997
Calcium pectate beads	Gas-lift	Smogrovicová and others 1997
κ-Carrageenan beads	Gas-lift	Mensour and others 1996
Ceramic beads	Fixed-bed	Inoue 1995
Gluten pellets	Fixed-bed	Bardi and others 1997
Polyvinyl alcohol beads	Gas-lift	Smogrovicová and others 2001
Porous glass beads	Fixed-bed	Virkajärvi and Krönlof 1998
Porous chitosan beads	Fluidized-bed	Unemoto and others 1998, Maeba and others 2000
Silicon carbide rods	Monolith reactor	Andries and others 1996
Spent grains	Gas-lift	Brányik and others 2002
Wood chips	Fixed-bed	Linko and others 1997, Kronlöf and Virkajärvi 1999

fermentors where high mass transfer restrictions, accumulation of carbon dioxide, nonuniform temperature profiles, flow channelling, and stagnant zones were observed during primary fermentation. Therefore, different approaches for the adaptation of immobilized systems were investigated in order to correct the final beer quality. The crucial elements were cell carrier selection and bioreactor design.

Packed-bed reactors have been selected for the production of alcohol-free or low-alcohol beers and for enhanced flavor maturation using immobilized cells. In these applications, conditions are anaerobic and yeast growth is limited. Preformed carrier materials are selected. Immobilization of cells can be by adsorption (e.g., DEAE–cellulose beads) or a combination of adsorption and entrapment (e.g., porous glass beads). These carrier materials need to be mechanically strong to withstand the high pressures in packed-bed reactors.

Recently, cheap carrier materials have been investigated as the use of these materials avoids the costly regeneration of used immobilization matrices. Wood chips have shown good performance and the use of this material has reduced the total investment cost by one-third compared to more expensive carriers (Kronlöf and others 2000).

An alternative preformed support material, based on spent grains, has been proposed recently for yeast cell immobilization for primary beer fermentation in an air-lift bioreactor (Brányik and others 2001, 2002, 2004a,b). This is an interesting carrier for cell immobilization, because spent grains are a waste byproduct of the brewing process.

Applications of Immobilized Cell Technology (ICT) in the Brewing Industry. ICT processes have been designed for different stages in the beer fermentation process, including wort acidification, bioflavoring during the secondary fermentation, primary fermentation, and fermentations for the production of alcohol-free or low-alcohol beers (for recent reviews see Branyik and others 2005; Nedovic and others 2005). The most challenging and most complex application is the combined main and secondary fermentations. A further major challenge to successfully apply ICT on an industrial scale is the control and fine-tuning of the flavor profile during a combined primary and secondary fermentation, as many parameters can have an influence on flavor formation (Willaert and Nedovic 2006).

FLAVOR MATURATION OF GREEN BEER. The objective of flavor maturation is the removal of diacetyl and 2,3-pentanedione, and their precursors α-acetolactate and α-acetohydroxybutyrate. The conversion of α-acetohydroxy acids to the vicinal diketones is the rate-limiting step. This reaction step can be accelerated by heating the beer – after yeast removal – to 80–90°C over a couple of minutes. The resulting vicinal diketones are subsequently reduced by immobilized cells into their less-flavor-active compounds.

The traditional maturation process is characterized by a near-zero temperature, low pH, and low yeast concentration, resulting in a very long maturation period of 3 to 4 weeks. Using immobilized cells, this long period can be reduced to 2 h. An ICT maturation process using a packed-bed bioreactor with DEAE–cellulose beads has been successfully integrated in the Synebrychoff Brewery (Finland) for the treatment of one million hL per year (Pajunen 1995). Alfa Laval and Schott Engineering developed a maturation system based on porous glass beads (Dillenhöfer and Rönn 1996). This system has been implemented in several breweries in Finland (Hyttinen and others 1995), Belgium, and Germany. The German company Brau & Brunnen has also shown an interest in the Alfa Laval maturation technology. In 1996, a 30,000 hL/year pilot-scale system was purchased and installed in their plant (Mensour and others 1997). The Alfa Laval maturation system has been implemented in a medium-sized German brewery (Schäff/Treuchtlingen) (Back and others 1998). The obtained beers yielded overall good analytical and sensorial results.

PRODUCTION OF ALCOHOL-FREE OR LOW-ALCOHOL BEER. The classic technology to produce alcohol-free or low-alcohol beer is based on the suppression of alcohol formation by arrested batch fermentation (Narziss and others 1992). However, the resulting beers are characterized by an undesirable wort aroma, because the wort aldehydes (especially 3-methylthiopropion aldehyde) have only been reduced to a limited degree (Collin and others 1991; Debourg and others 1994; van Iersel and others 1998; Perpète and Collin 1999; Van Nedervelde and others 1999). The reduction of these wort aldehydes can be quickly achieved by a short contact time with the immobilized yeast cells at a low temperature without undesirable cell growth and ethanol production. A disadvantage of this short contact process is the production of only a small amount of desirable esters.

Controlled ethanol production for low-alcohol and alcohol-free beers has been successfully achieved by partial fermentation using DEAE–cellulose as the carrier material, which was packed in a column reactor (Collin and others 1991; Van Dieren 1995). This technology has been successfully implemented by Bavaria Brewery (The Netherlands) to produce malt beer on an industrial scale (150,000 hL/year) (Pittner and others 1993). Several other companies, such as Faxe (Denmark), Ottakringer (Austria), and a Spanish brewery, have also implemented this technology (Mensour and others 1997). In Brewery Beck (Germany), a fluidized-bed pilot-scale reactor (8 hL/day) filled with

porous glass beads was used for the continuous production of nonalcohol beer (Aivasidis and others 1991; Breitenbücher and Mistler 1995; Aivasidis 1996). Yeast cells immobilized in silicon carbide rods and arranged in a multichannel loop reactor (Meura, Belgium), have been used to produce alcohol-free beer on a pilot scale by Grolsch Brewery (The Netherlands) and Guinness Brewery (Ireland) (Van De Winkel 1995).

Nuclear mutants of *S. cerevisiae* that are defective in the synthesis of tricarboxylic acid cycle enzymes, that is, fumarase (Kaclíková and others 1992) or 2-oxoglutarate dehydrogenase (Mockovciaková and others 1993), have been immobilized in calcium pectate gel beads and used in a continuous process for the production of nonalcoholic beer (Navrátil and others 2000). These strains produced minimal amounts of ethanol and they were also able to produce much lactic acid (up to 0.64 g/dm^3).

PRODUCTION OF ACIDIFIED WORT USING IMMOBILIZED LACTIC ACID BACTERIA. The objective of this technology is the acidification of the wort according to the "Reinheidsgebot", before the start of the boiling process in the brewhouse. An increased productivity of acidified wort has been obtained using immobilized *Lactobacillus amylovorus* on DEAE–cellulose beads (Pittner and others 1993; Meersman 1994). The pH of the wort was reduced below a value of 4.0 after contact times of 7–12 min using a packed-bed reactor in downflow mode. The produced acidified wort was stored in a holding tank and used during wort production to adjust the pH.

CONTINUOUS PRIMARY FERMENTATION. The Japanese brewery Kirin developed a multistage continuous fermentation process (Inoue 1995; Yamauchi and others 1994; Yamauchi and Kashihara 1995). The first stage is a stirred tank reactor for yeast growth, followed by packed-bed fermenters, and the final step is a packed-bed maturation column. The first stage ensures adequate yeast cell growth with the desirable free amino nitrogen consumption. Ca-alginate was initially selected as the carrier material to immobilize the yeast cells. These alginate beads were later replaced by ceramic beads ("Bioceramic®"). This system allowed beer to be produced within three to five days. This pilot-scale (100 hL) system was operational over two years in a restaurant brewery on Saipan Island (Northern Mariana Islands). The production scale was 5 hL/day, and beer of acceptable quality was produced.

The engineering company Meura (Belgium) developed a reactor configuration with a first stage with immobilized yeast cells where partial attenuation and yeast growth occurs, followed by a stirred tank reactor (with free yeast cells) for complete attenuation, ester formation, and flavor maturation (Andries and others 1996; Masschelein and Andries 1995). Silicon carbide multichannel porous rods (60% void volume) are used in the first reactor as immobilization carrier material. The porous rods are seeded with yeast cells and perfused in parallel with a recirculating feed medium. The stirred tank (second reactor) is continuously inoculated by free cells that escape from the first immobilized yeast cell reactor. The system is characterized by a simple design, which can easily be scaled up. Disadvantages are the relatively high cost of silicon carbide matrices, and lower cell growth and specific productivity in this system as compared to free cells. The immobilized reactor system has been found to be stable over a period of more than six months, producing ale and lager beer of excellent quality (Andries and others 2000).

Labatt Breweries (now InBev, Canada), in collaboration with the Department of Chemical and Biochemical Engineering at the University of Western Ontario (Canada), developed a continuous system using κ-carrageenan immobilized yeast cells in an airlift reactor (Mensour and others 1995, 1996, 1997; Pilkington and others 1999). Pilot-scale

(50 L) research showed that full attenuation was reached in 20–24 h with this system compared to 5–7 days for the traditional batch fermentation. Although the flavor profiles of the beer produced using ICT and the batch-fermented beer differed somewhat, a taste panel judged the immobilized cell product to be acceptable and, overall, similar to the conventional control fermentation product.

Hartwell Lahti and VTT Research Institute (Finland) developed a primary fermentation system using ICT on a pilot scale of 600 L/day (Kronlöf and others 1989; Kronlöf and Virkajärvi 1999). Woodchips were used as the carrier material. The results showed that fermentation and flavor formation were very similar compared to a traditional batch process, although the process time was reduced to 40 h.

Synebrychoff Brewery (Finland), in collaboration with Guinness, GEA Liquid Processing Scandinavia, and Cultor Corporation of Finland, developed a new ICT process in which the concentration of carbon dioxide is controlled in a fixed-bed reactor in such a way that the CO_2 formed is kept dissolved, and is removed from the beer without foaming problems (Andersen and others 1999). DEAE–cellulose was used as the carrier material. High-gravity beer of acceptable quality has been fermented over 20 h at a capacity of 50 L/h. Later, wood chips were used as carrier material (Pajunen and others 2001; Tapani and others 2003). Good quality beer and a constant flavor profile were achieved with a production time of 20–30 h. In a pilot plant (reactor volume of 10 hL, 12 hL/day), the fermentation process and flavor formation could be controlled by using the feed rate, recirculation rate, and fermentation temperature as control variables (Pajunen and others 2001).

A gas-lift bioreactor system, which was introduced in beer fermentation experimental studies by the research group at Belgrade University, is another promising concept for main beer fermentation (Nedovic and others 1993). Hydrogel carriers like alginate, carrageenan, and pectate demonstrated limited mechanical stability and resistence when applied in packed-bed reactors. However, these porous matrices present a good solution for yeast immobilization in gas-lift bioreactors. Laboratory- and semi-pilot-scale bioreactor systems were developed with alginate microbeads (0.8 mm in diameter) and lens-shaped PVA particles ("LentiKats^{TM}") as carriers for yeast cells (Nedovic and others 2004, 2005). Full beer attenuation in these experiments was reached within 7.5–20 h depending on solid loading (10–40%). The final beers had the desired sensory and analytical profiles.

A combined continuous immobilized system for primary fermentation and maturation has been developed and characterized (Smogrovicová and others 2001). The primary fermentation was carried out in a gas-lift bioreactor, the maturation in a packed-bed reactor. The green beer was heat-treated (to accelerate the conversion of α-acetolactate to diacetyl) before it was fed to the maturation reactor. Yeast cells were entrapped in lens-shaped PVA particles and used to perform the primary fermentation as well as the maturation. The system was stable for 2 months at a residence time of 24–36 h. The produced beer was characterized by a composition and flavor profile that was similar to that of beer produced by a classical batch fermentation.

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21

Manufacture of Whisky

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21.1 Introduction 507
21.2 Scottish Whisky 508
21.2.1 Malt Whisky 508
21.2.1.1 Malting 508
21.2.1.2 Mashing 509
21.2.1.3 Fermentation 510
21.2.1.4 Distillation 510
21.2.1.5 Maturation 513
21.2.1.6 Bottling 514
21.2.2 Grain Whisky 515
21.2.3 Blended Whisky 516
21.3 Irish Whiskey 517
21.4 American Whiskey 517
21.5 Canadian Whisky 518
21.6 Japanese Whisky 518
21.7 Conclusions 519
References 520

21.1 INTRODUCTION

One of the world's greatest alcoholic drinks is whisky (whiskey). The term "whisky (whiskey)" was derived from the Gaelic "uisge beatha", equivalent to the Latin term "aqua vitae", meaning "water of life". It was invented by accident by alchemists before the eleventh century and they gave the drink this name (Gavin 1997).

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It was reported that the Irish were drinking a form of whisky in the twelfth century, and Henry of England said that the distillation of spirit "usquebaugh" was well established in Ireland when he invaded there in 1172 (Daiches 1995). It is clear that distilling was certainly then brought from Ireland to Scotland in the early Middle Ages, although it seems to have been known in the Far East at a very early date among alchemists (Kippple and Ornelas 2000). By the end of the fifteenth century, whisky was well established in Scotland, with the first record of a commercial transaction involving the supply of whisky (aqua vitae) in the year 1494. The manufacture of whisky was brought to the United States of America in the seventeenth to eighteenth centuries and further developed to manufacture in Canada. In the early twentieth century, whisky manufacture was also brought to Japan. These five countries (Ireland, Scotland, the United States, Canada, and Japan) produce about 95% of the whisky in the world and, therefore, they are known to be the five largest international whisky-distilling countries (Tsuchiya 2000; Bathgate 2003). Many other countries are also producing whisky (Murphy 1978).

The appellation of whisky, spelled without an "e" in Scotland, Canada, and Japan, but with an "e" in Ireland and the United States, is the generic name for a distilled product made from saccharified and fermented cereal extracts.

21.2 SCOTTISH WHISKY

The word "Scotch" is, of course, a contraction of "Scottish whisky" and was first used in the mid-nineteenth century (Benitah 2002). Scotch malt whisky was well established in Scotland by the end of the fifteenth century, and approximately 700,000 kiloliters (kL) is produced per year. It is consumed in over 200 countries worldwide (Tsuchiya 2006a). There are three types of whisky in Scotland, malt whisky, grain whisky, and blended whisky.

21.2.1 Malt Whisky

Scottish malt whisky is produced by using barley, water, and peat as the raw materials. Barley locally grown in the northeast of the country was originally used. However, some of the greatest Highland distilleries today also use imported barley. Large grain two-row barley, with high starch and low protein content, is preferred.

21.2.1.1 Malting. Malting is the first step in making whisky. Malt is barley that has been persuaded to germinate by soaking in water and has then been dried by the application of heat (Daiches 1995).

The "Steeping" or soaking period in the tank is usually 2 to 3 days. The water is drained off and the grain spread out to a depth of 20–30 cm on a concrete or tiled floor in the malting house. The water content of the barley rises to approximately 43% by this soaking or steeping process. Barley begins to germinate on the malting floor and generates energy that raises the temperature. The barley is turned with rakes and wooden shovels by maltmen every 4 or 6 h in order to maintain approximately 16°C temperature and to prevent growing roots tangling each other. After 7 to 10 days, the growing stem of the barley becomes one-half to five-eighths of the length of the seed (Tsuchiya 2000).

To stop the germination, green malt is transferred to the drying kiln, which has a unique chimney (Fig. 21.1). The floor of the kiln is a drain board made of perforated iron or wire mesh. The green malt is spread on this floor at a depth of 70 cm to 1 m, depending on the design of the kiln, and dried in smoke rising from a peat fire below the floor (Tsuchiya

Figure 21.1 *Drying kiln, with its unique chimney.*

2000). Figure 21.2 shows a sample of the peat used to dry malt. Besides peat, coal and coke are often used as fuel and the barley is dried for 40–55 h until the moisture content becomes 3–4%. The peat smoke gives a special smoky flavor to the green malt and to the final product: mature whisky. A growing number of distilleries no longer do their own malting, but buy their malt ready-made from maltsters, peated to the desired degree. The kiln building is only kept as a symbol of such distilleries (Simpson 1994).

21.2.1.2 Mashing. The dried malt is moved to the malt mill and ground to fine grist consisting of husk, grits, and flour in the ratio 2 : 7 : 1 (Tsuchiya 2000). The malt grist is placed in a container known as mash tun and hot water is added. The shape and size of mash tuns vary

Figure 21.2 *Sample of peat used to dry malt.*

Figure 21.3 *Mash tun.*

and they are made from stainless steel, copper, or cast iron, usually with a lid (Fig. 21.3). Mashing or extraction of the malt is carried out three or sometimes four times with hot water, each time at a different temperature, ranging from about 60°C for the first process to 100°C for the last (Daiches 1995). Mashing with hot water dissolves the starch from the malt and also activates the amylase that decomposes the starch to maltose.

Wort (the resultant liquid) is drawn off from the base of the mash tun through the finely slotted bottom, cooled to around 20°C, and passed into fermentation vessels, or washbacks. The residual solids (draff) are removed from the floor of the mash tun and used as cattle feed (Arthur 2003).

21.2.1.3 Fermentation. The washback is made of wood or stainless steel and has a lid that incorporates a rotating blade that prevents foaming over the sides (Fig. 21.4). When the washback is two-thirds full, yeast is added in liquid or solid form to the wort and fermentation takes place at 17–35°C for 48 h (on average). The inoculated yeast converts maltose into glucose, and then alcohol and carbon dioxide (CO_2) are produced from glucose. Yeast also produce small amounts of other compounds such as a wide range of esters, aldehydes, acids, and higher alcohols. Many of these are flavor elements. The fermented wort (wash) is a sweet peaty beer-like liquid with an alcoholic content of 7–8% (Tsuchiya 2000).

21.2.1.4 Distillation. The next process, distillation, is what actually produces the whisky. Scottish malt whisky has been traditionally distilled in onion-shaped stills (pot stills), which are large copper kettles with narrow necks called lyne arms or lyne pipes that curve and enter the condenser, often located in the open air outside the still house (Simpson 2003). The shape and size of the pot still affect the quality of the whisky produced. Stills come in three basic designs, the "onion" being the most common, and the "boil-ball" and "lantern" shape (Fig. 21.5).

The way these designs are interpreted – as to capacity, height, method of heating, angle of the lyne arm, and so on – differs from one distillery to another, and varies the quantity

Figure 21.4 *Washback.*

of volatiles that will end up in the spirits (MacLean 2002; Nicol 2003). Traditional direct firing either by coal or gas to heat up the pot still is retained by many distilleries. However, a large number of distilleries have now switched to heating the stills by means of internal steam exchangers (Simpson 1994). The direct-fired wash stills have "rummagers" (revolving arms) to prevent solids in the wash sticking to the bottom of the still and scorching (Simpson 1994).

The area of copper that comes into contact with the wash and low wines is another consideration in still design. Copper dissolves easily and has a decisive influence on the quality of the spirit, as it removes sulfury or vegetable aromas by a chemical reaction (MacLean 2002).

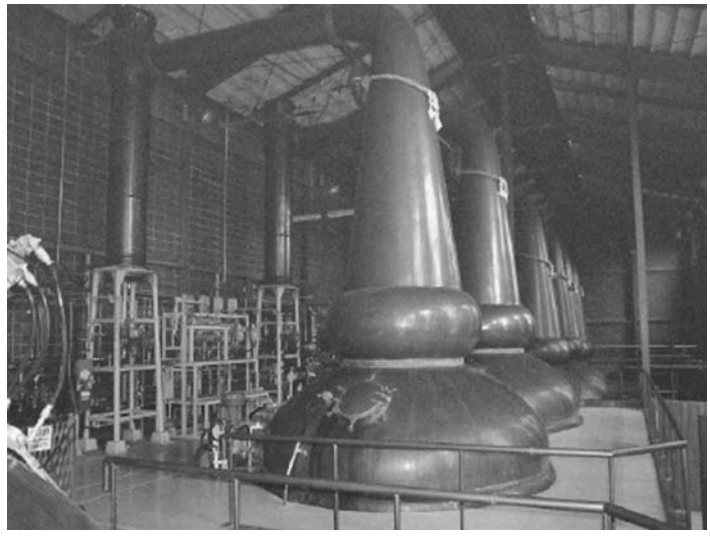

Figure 21.5 *Still.*

Because malt whisky is usually produced by double distillation, every distillery must have at least two such stills. Occasionally a third still is installed. A distillery with only one still operates the functions of wash and low wine stills in sequence.

For the first distillation, the fermented wash passes through the wash charger, into the wash still (or singling still). The charged volume is between half and two-thirds capacity, in order to allow for expansion of the wash and the froth that builds up as it is heated (MacLean 2002). When the wash in the still is heated, the alcohol (ethanol) vaporizes at a fairly low temperature (78.3°C), and passes up the neck of the still and down through the worm (coiled copper pipe) within a water jacket (worm tub or condenser). The distillate from the wash, low wine, consists of 21–24% by volume of alcohol (ABV). The first distillation is finished when the liquid left in the wash still is of no more than 1% ABV. At the end of the run, about a third of the wash will have become low wine (MacLean 2002).

As the low wine contains a large number of other volatile alcohols and oils, it must be further purified by a second distillation in the low wine still (or double/second still), which is smaller than the wash still. During the second distillation, its progress is carefully monitored by the stillman, who starts to test the spirit as soon as the condensed vapors pass through the spirit safe (Fig. 21.6).

The first distillate, called *foreshots* or *head*, is tested for the presence of impurites. When water is added, it will turn cloudy. This is called the demisting test. Foreshots are of high alcohol strength (75–80% ABV) and pungent, owing to the impurities they contain (Simpson 2003), such as the aldehyde, esters, furfurals and other compounds of hydrogen, oxygen, and carbon formed in the process of distilling the wash and known generally as congeneric, congeners, or congeries. However, they also give pot-still malt whisky its special bouquet and flavor (Daiches 1995). The foreshots are directed into the low wine charger to be redistilled with the next batch of low wines. The stillman continues to test the spirit in the spirit safe by adding water at regular intervals and checking the specific gravity. As soon as the spirit ceases to turn cloudy with the addition of water, it

Figure 21.6 *Monitoring of distillation as soon as vapors pass through the spirit safe.*

is immediately directed to the spirit receiver for collection (Arthur 2003). The spirit directed to the spirit receiver is called *hearts* or *middle cut* which consists of about 70% by volume of alcohol.

The amount of the hearts is approximately one-third of the spirit distilled. When the spirit starts to weaken, this weakened spirit, called *feints*, *after-shots*, or *tail*, is run into the feints receiver. The stillman stops collecting the spirit at the point when the alcohol content is as high as 69% by volume of alcohol for the lighter whisky character and as low as 60% for the heavier whisky character (MacLean 2002). The breadth of the cut has a profound effect on the quality of the final product. The collected feints and foreshots will be added to the next low wine for distillation. The residue in the low wine still, *spent lees*, is of no use or value and goes to waste (MacLean 2002).

21.2.1.5 Maturation. The immature spirit, called *new pot* or *new spirit*, is piped to the filling station, where it is diluted with water until its strength is reduced to 63.5% by volume of alcohol (Tsuchiya 2000). This diluted spirit is stored in casks made either from American white oak (*Quercus alba*) or European oak (*Quercus robur*) for maturation. Oakwood is thought to be desirable for maturing whisky because of its chemical complexity. The casks are secondhand, in that they have been used to store either sherry or bourbon, and in rare cases port or other wines.

Maturation of new spirits is conducted in casks stored in warehouses called "dunnage" warehouses for at least three years undisturbed. After that they can be legally called scotch whisky.

Bottling of "single malt" or "deluxe blend" whisky uses spirits matured in the cask for at least 10 to 15 years (Arthur 2003). However, there is always the danger of the whisky acquiring a slightly "woody" flavor after 15 years (Daiches 1995). Whisky maturation is influenced by factors such as the nature and history of the cask, the style of warehouse and its location, the outside microclimate of casks, and the maturation period. Oakwood facilitates oxidation, which removes harshness, increases fruitness and adds complexity (MacLean 2002). All trees suitable for making casks must be over 80 years old. Oak casks that have previously been filled with sherry give a splendid mellowing effect (color, softness, richness, and smoothness) to the mature whisky, and bourbon casks give a typically vanilla-like aroma. The process of charring of the inside walls of bourbon casks also contributes to the flavor of malt whisky. This releases vanillin into the spirits and assists in removing undesirable off-notes (Figs 21.7 and 21.8). Sherry casks are toasted rather than charred.

A small loss of spirit is expected during maturation. The smaller the cask, the faster the maturing and the larger the amount of whisky lost through absorption by the porosity of the cask wood. The degree of humidity in the warehouse where the casks lie during the maturation also affects the degree of loss of maturing whisky both in its volume and its strength. The higher the humidity, the more it loses strength (by about 4–5% ABV in ten years), and the drier, the more it loses volume (Daiches 1995). This is known as the "angel's share", a loss of 1–3% every year (Tsuchiya 2000).

Alcohol content decreases from 63.3% to around 60% and the volume becomes 80% of the initial filling after ten years' maturation. The longer whisky is matured in the cask, the more changes of color and flavor will take place. It should be noted that each cask of malt whisky from the same distillery tastes different from its neighbor, even if they came from the same still run (Simpson 1994).

Figure 21.7 *Casks.*

21.2.1.6 Bottling. Before bottling, whisky is further diluted with water until its strength is reduced to 40–60% by volume of alcohol. Soft water, free from organic and mineral impurity, is preferred for whisky production. Whisky is usually filtrated through a cellulose filter to remove fatty particles at a temperature between 4 and 10°C prior to being run into bottles (Simpson 1994). Once run into the bottles, whisky does not further mature, but some chemical changes will occur.

Single malt whisky is the product of a single distillery. When bottled, the single malt may include whisky from several years' production from the same distillery (Arthur 2003). The age shown on the bottle label means the length of the youngest whisky's maturation period. A malt whisky that is bottled from an individual cask is called single cask whisky. A vatted malt whisky is a product that is "married" together with various malt whiskies from several distilleries. A vatted malt whisky is labeled

Figure 21.8 *Casks.*

"pure malt" or "scotch malt whisky". It is impossible to determine the optimum age for whisky in general terms; so much depends on the individual case.

There are about 110 malt whisky distilleries in Scotland and about 80 to 90 distilleries are now in operation (K-Writer's Club 1998; Futagani 2000; Tsuchiya 2002). Scotch whisky is traditionally divided into Highland malts, Lowland malts, Campbeltown and Islays (Hirazawa 1990; Fikunishi 1992; Hashiguchi 2002; Hashiguchi 2003).

21.2.2 Grain Whisky

In Scotland, when whisky is produced largely from maize with a small amount of malted barley that is not dried over peat fires, it is called Scottish grain whisky. In some cases, unmalted barley is also used with the maize. The unmalted cereal is crushed and then pressure-cooked in batch cookers to gelatinize the starch so that it can be released and solubilized. Wet ground malted barley is added in an amount one-fifth of the amount of unmalted cereal, followed by hot water, and the mixture is stirred (Daiches 1995). Amylase from malted barley converts the starch to fermentable sugars, maltose. Although many distillers retain this system, there is an increasing use of continuous operation for the cooking and conversion process (Simpson 1994).

Fermentation of the wort is carried out in the same way, by the action of inoculated yeast, as in the case of making malt whisky. The fermented wort, *wash*, is pumped into the patent still or Coffey still, which distillates wins a different system from distillation using a pot still. The patent still was first invented by Robert Stein in 1826 and was improved by Aeneas Coffey in 1831. With the invention of the patent still, making whisky has become quicker, cheaper, and occurs in greater volumes. The product has no peaty flavor, for the malt had not been dried over peat fires (Daiches 1995; Tsuchiya 2000). Figure 21.9 shows a Coffey still made in Scotland, which is now running in Japan.

The patent still basically consists of two linked copper columns, 12–15 m high; these are called the analyzer and the rectifier. Each is divided horizontally into tens of chambers by perforated copper plates. Steam is led into the analyzer and proceeds up the analyzer and then through the linking pipe into the rectifier. The wash is continuously pumped into the rectifier, traveling down in a coiled copper pipe and then via the connecting wash pipe into the top of the analyzer. The heated wash moves down, chamber by chamber in the analyzer, and all the alcohol contained is vaporized before the wash reaches the base of the analyzer. The steam and alcohol vapors, which rise up through the analyzer, travel down the connecting vapor pipe (which emerges from the top of the analyzer) into the base of the rectifier. As the alcohol vapor and the steam rise up to the rectifier, chamber by chamber, the new incoming wash, coming down the rectifier in the winding pipe, is then cooled, and condensation takes place (Daiches 1995; Brander 1996; Hashiguchi 2002).

Around 95% alcohol can be obtained when the vapor is drawn off through a cooling worm to the stainless steel spirit receivers. Alcohol strength for making whisky is regulated below 94.8% by the law of Scotland (Tsuchiya 2000). The spirit is then diluted with water to around 70% by volume of alcohol, prior to being run into secondhand oak casks that were previously used for the maturation of malt whisky. The spirit-filled casks are left undisturbed in the warehouse for at least three years, the same as malt whisky (Tsuchiya 2000).

The grain whisky, being nearer to a neutral spirit than pot-still malt whisky, takes less time to mature and changes less in the maturing process. There are two types of grain whisky; one

Figure 21.9 *Coffey still.*

is a single-grain whisky produced in one distillery, and the other is that produced by vatting whiskies distilled in several distilleries. The age shown on the bottle label indicates the length of the youngest whisky's maturation period. Only eight grain distilleries are in operation; seven of these are located in Lowland Scotland (Benitah 2002).

21.2.3 Blended Whisky

A blended whisky is created from both single malts and grain whisky. Andrew Usher, of Edinburgh, pioneered blending in the early 1860s (Tsuchiya 2000). Blends are central to the Scotch whisky market, outselling malt whiskies at a ratio of around nine to one.

Blending can involve combining around 20 to 50 different malt whiskies with two to five grain whiskies to produce mild whisky with a well-balanced flavor (Daiches 1995; Hashiguchi 2002). It provides the distiller with an opportunity to create a product with unique and recognizable flavor attributes. It also enables product consistency. The proportion is 60% grain to 40% malt. The proportion of grain has risen since the early days of blending, when the ratio was more likely to be 50:50 (Daiches 1995). Deluxe blended whiskies generally contain a higher ratio of malt to grain than standard blends (Gavin 1997).

The blended whisky is left undisturbed in oak casks (generally plain oak) for 6 to 12 months in order to optimize the product quality. Some blenders "marry" the malts

and the grains separately and bring them together only in bottling. Prior to bottling, the whisky is usually diluted to market strength, caramel is added to ensure continuity of coloring, and finally the spirit is filtered to prevent cloudiness from developing when water is added by the consumer (Jaganathan and Dugar 1999). As for blends, the age (if any) stated on the label is the age of the youngest whisky in the blend.

21.3 IRISH WHISKEY

Only whiskey produced in Ireland can be called Irish whiskey. It is well known that whiskey distilling originated in Ireland. The Irish were drinking a form of whiskey in the twelfth century and their skills in whiskey-making were taken to Scotland (Daiches 1995; Gavin 1997; Hashiguchi 2002). The extra "e" in the name is traditional, but gives no special meaning (Benitah 2002).

Malt and unmalted barley are what are needed to produce the Irish malt whiskey. However, much of the current productions of Irish whiskey use oats, wheat, and rye with barley.

The size and design of Irish pot stills are quite different from Scottish pot stills. Irish stills have volumes in the range 50,000–150,000 L, whereas Scottish stills have volumes ranging from 2000 to 30,000 L. Irish stills are attached to purifiers that remove congenerics such as aldehydes, esters, furfurals and other compounds of hydrogen, oxygen, and carbon formed in the process of distilling the wash (Daiches 1995).

Irish whiskey also differs from Scotch whisky in the distillation profiles. Two separate fractions of the low wines are collected from the wash still based on differences in alcohol concentration. The weak low wines are distilled in a low wines still to obtain further fractionation, strong and weak feints. The weak feints are redistilled with the next batch of weak low wines, and the strong feints are added to the strong low wines for the third distillation.

An alcohol concentration of about 80% ABV in the new spirit is much higher than that in Scottish malt (Benitah 2002). New spirits are first reduced to 63.5% ABV before filling into secondhand bourbon, rum, and sherry casks for at least three years (usually five to seven years). These straight Irish whiskeys are then blended with grain whiskey before bottling.

The more temperate climate of Ireland produces whiskeys with a slightly spicier taste and a crisp finish (Arthur 2003). Three distilleries are now in operation (Hashiguchi 2003). Although the market for Irish whiskey is limited, it is internationally recognized as a distinct generic brand (Bathgate 2003).

21.4 AMERICAN WHISKEY

American bourbon whiskey originates from the whiskey produced in the Bourbon County in the state of Kentucky – thus the name "bourbon", in short. However, it is now common to call all whiskeys produced in the United States as bourbon. By law, bourbon must be produced from a mash of not less than 51% corn grain (usually between 70 and 90% corn), with some barley malt and rye or wheat (Takeuchi 2000; Benitah 2002).

American whiskey applies a distinctive sour mash technique in its production. Inoculation of lactic acid bacteria prevents bacterial contamination. The residue (called backset or setback) of former distillation is added prior to yeast fermentation. If no sour mash is

added at the start of fermentation, the result is called sweet-mash straight whiskey (Benitah 2002). The distillation is carried out in beer stills (column stills or patent stills), which is common to nearly all American whiskeys.

Spirits are collected at relatively low alcohol concentrations, from 64 to 70% by volume. Therefore, various flavor components are also included, and the product has a distinct aroma.

The spirit is matured for at least two years in new white-oak barrels that have been charred. Charring has been used as a means of disinfecting barrels since the early nineteenth century. In bourbons, the definite characteristics are the tones of vanillin and caramel that the whiskey takes from the wood. It is bottled at over 40% by volume of alcohol. Nine distilleries of Kentucky bourbon are now in operation, producing various brands (Tsuchiya 2006a).

Tennessee whiskey, legally classified as bourbon, is produced in the state of Tennessee using the same method as Kentucky bourbon. The new spirit is filtered through maple charcoal, prior to maturation in the barrel. It has a mellow taste.

Rye whiskey is made from a mix of grains containing a minimum of 51% rye, and straight rye whiskey is matured for at least two years in the charred white-oak barrels.

Corn whiskey is made from a mixture of grains containing a minimum of 80% corn. The product matured for at least two years in oak barrels is called straight corn whiskey (Hasiguchi 2002).

A neutral grain spirit is also made in the United States and it is exclusively used in blends. In the United States, alcohol content is expressed in terms of proof, not as a percentage of alcohol by volume (ABV). To convert proof to % ABV, simply divide by two (Benitah 2002).

21.5 CANADIAN WHISKY

Canada also produces a whisky, the Canadian whisky. Hiram Walker, in 1858, founded the first distillery, and the product was launched as Canadian Club brand in 1884 (Benitah 2002). It is produced from corn, rye, and barley malt.

When rye is used at levels over 51%, its product can be named “rye whisky”. There are two types of Canadian whisky: one is the flavoring whisky that is produced mainly from rye and the other is the base whisky that is made mainly from corn. Both are distilled in column stills. Flavoring whisky is matured in a charred barrel (new or old) and the base whisky is matured in regenerated bourbon barrels. Both are matured in small barrels (smaller than 180 Ls) for at least three years, then blended to make various brands.

Canadian whisky is characterized as flavory, light, and mild, as compared to other whiskies (Fukunishi 1992). Canadian whisky has been industrially developed to supply mainly the U.S. market. Today, there are a dozen or so distilleries across the country (Benitah 2002).

21.6 JAPANESE WHISKY

Scottish/Irish immigrants did not found the whisky industry of Japan, but Japanese and Scottish distillers have strong ties. In the 1920s, the first Japanese distillers were trained in Scotland and took their new-found knowledge to traditional sake distilleries in Japan (Arthur 2003; Bathgate 2003). The first Japanese distillery was established in 1923 in the valley of Yamazaki on the outskirts of Kyoto, and launched its first whisky in 1929. This

Figure 21.10

whisky was a blended product of malt and unmatured neutral spirit. A matured grain whisky made from North American maize has now replaced the neutral spirit in blending.

By buying into Scottish distillery operations, the Japanese distilling industry has renewed and strengthened its bonds with Scotland. Other Japanese businesses and trading houses have also participated in the growth of whisky distilling, either by setting up joint-venture companies or by importing Scotch whisky. They set out to produce the best raw materials, distilling technology, and maturing conditions required in the production of the "Scotch malt" type of whisky.

The landscape of the northern island, Hokkaido, is very similar to that of the Highlands of Scotland, with peat bogs, mountains, and cool, fresh streams, which flow over granite rocks (Simpson 1994; Bathgate 2003). Yoichi distillery of Nikka Company on the island of Hokkaido northwest of the city of Sapporo makes the most peaty of Japanese single malts (Benitah 2002).

Malt is imported mainly from Scotland, but with an increasing amount coming from Australia. Stainless-steel washbacks with cultured yeast are used for malt whisky fermentation, and distillation is conducted in traditional copper wash and spirit pot stills. North American maize used in the making of grain whisky is mashed and distilled conventionally. Malt and grain whiskies are matured in oak casks before blending, the same way as in Scotland.

The most popular brands of Japanee whiskies are blends. However, the companies Suntory, Nikka, and KIRIN have been successfully in developing single malts (Fig. 21.10). They are indeed whiskies in their own right (Benitah 2002). Seven distilleries are now in operation and the total volume of Japanese whisky production reached 70,500 kL in 2003 (Tsuchiya 2006a,b).

21.7 CONCLUSIONS

Whisky is the world's most consistently successful and popular alcoholic drink. According to documentary records, it was first commerced in Scotland in 1494. Scotland, Ireland, the United States, Canada, and Japan are now the five major whisky-producing countries. Each country has developed its own style of whisky into something approaching a fine art. Each country's particular style will remain essentially unchanged and be eternally inherited by successive generations.

Recently, "light" whiskies that have a lighter flavor, color, and body have been promoted, mainly in the U.S. and Japanese markets. The proportion of single malts to blends consumed, even in Scotland, is very small; nevertheless there is a steady rise in the availability and consumption of single malts both in Britain and in certain other countries (Daiches 1995). The promotion of single malts in the whisky market has been accompanied by the development of distillery visitor centers, complete with guide tours.

The consumption of small amounts of whisky is now considered to be healthy. Its aroma produces an effect of relaxation and contains polyphenols that act as antioxidants important in the body for the protection against free-radical damage (Goldberg and others 1999; Tsuchiya 2006a). The marketer must continue to follow consumer trends and make efforts to meet their needs.

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Section VI

Cereals: Rice and Noodles

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

22

Rice-Based Products

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22.1 Introduction 523
22.2 Rice Processing 525
22.3 Parboiled Rice 526
22.4 Brown rice and Germinated Brown Rice 526
22.5 Milled Rice 528
22.6 Enriched or Fortified Rice 529
22.7 Expanded Rice Products and Snacks 530
22.8 Broken Rice 530
22.9 Rice Flour 531
22.10 Hulls and Husk Applications 533
22.11 Bran Applications 533
References 534

22.1 INTRODUCTION

Rice (*Oryza sativa* L.) has been, throughout history, one of the most important foods in the human diet. It is likely that rice is the one grain that has fed more people in history than any other crop. Even today, rice grains sustain two-thirds of the world's population, although the contribution of rice is different in the developing and developed countries, and also the types of processing are quite different. Rice is the staple food of the Chinese and also 65% of the total population of India. According to the International Rice Research Institute (IRRI), rice will be the major source of food for 3.9 billion people by 2025. It is primarily consumed as milled rice, but there are also a number of products where rice is added as an

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TABLE 22.1 Top 10 Rice Producing and Consuming Countries.

Countries	Production (metric tonnes)	Countries	Human Consumption (kg per capita per year)
World	575,105,490	World	85.9
China	176,342,195	Myanmar	306.9
India	113,580,000	Vietnam	253.3
Indonesia	51,579,100	Bangladesh	245.4
Bangladesh	37,851,000	Cambodia	223.2
Vietnam	34,447,200	Indonesia	222.6
Thailand	25,610,900	Philippines	156.8
Myanmar	22,780,000	Thailand	153.8
Philippines	13,270,650	Nepal	152.8
Japan	11,111,000	India	125.0
Brazil	10,457,100	China	124.1

Source: FAOSTAT data, 2004

ingredient, conferring creaminess, crunchiness, and firmness. Moreover, rice-based products have often been a solution for consumers with allergenic problems.

Rice accounts for 29% of total cereal production, comparable to the production of wheat or corn. Nevertheless, its importance as a food cereal in the human diet has been calculated to be a little lower than wheat, although this assessment is reversed when only developing countries are taken into account. Rice cultivation is concentrated in developing countries, mainly in Asia, accounting for 91% of total world production (FAOSTAT 2004). China is the world's largest rice producer (31%) (Table 22.1), followed by India (20%), Indonesia (9%), and Bangladesh (7%). Rice cultivation is the principal activity and source of income in those countries, and also the central part of their diet, providing sustenance to the poor in many of those countries. Rice consumers are mainly located in Myanmar, followed by Vietnam, Bangladesh, Cambodia, and Indonesia. Rice

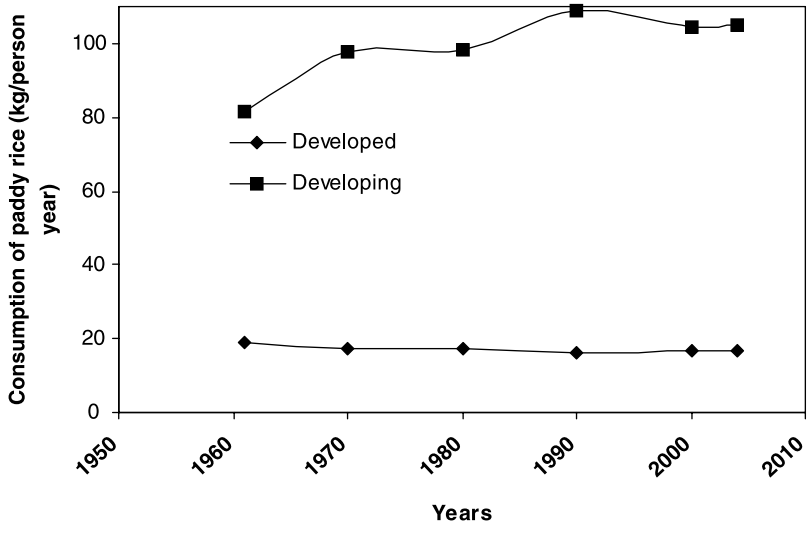

Figure 22.1 *Consumption of paddy rice in developed and developing countries.*

consumption in those countries greatly contrasts with that in the United States of America, where consumption drops to 14.1 kg per capita per year. This value is close to the average consumption of rice in the developed countries (16.1 kg per capita per year), in contrast with the 104.7 kg per capita per year of rice consumed in developing countries, which coincides with the low-income status of those countries (Fig. 22.1). In the last decade, rice consumption has experienced a steady decrease in developing countries, promoting the development of new and innovative rice-based products. In 2000, over 400 new products containing rice were launched to the market (Wilkinson and Champagne 2004), demonstrating the new initiatives for increasing rice consumption.

22.2 RICE PROCESSING

A simple flow diagram describing rice-based products is included in Figure 22.2. Rice from the field is harvested and threshed to produce the so-called paddy rice or rough rice, where the kernel is still within the hull or husk. Milling is the usual way to process rice and wheat. However, the term milling in the rice industry completely differs from the concept of wheat milling. Wheat is milled for obtaining flour, but milling of rice includes the steps of

1. Removing of the husk;
2. Stripping off the bran of the endosperm; and
3. Separating out broken kernels and other altered kernels.

All these processes drastically affect rice composition. Milling of rice is a very sophisticated process. Initially, paddy rice is cleaned through coarse screens to remove all straw, stones, and other foreign objects that are larger than the rice kernels. The same process is repeated using fine screens for removing small weed seed, sand, stones, and other objects

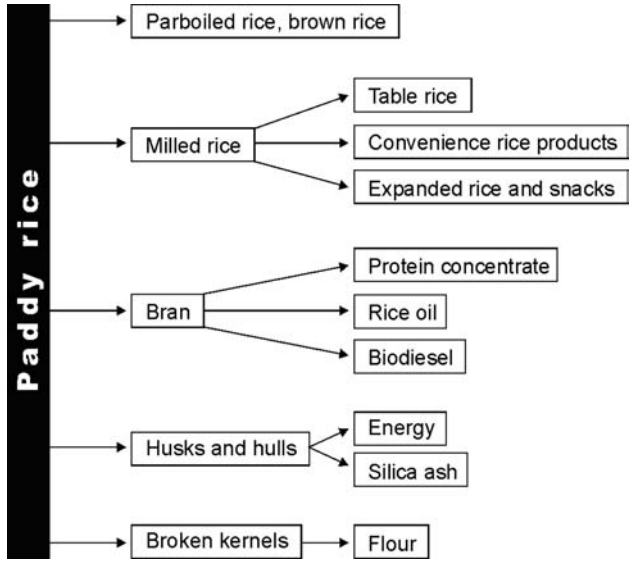

Figure 22.2

smaller than the rice kernels. Stones are separated from the rice kernels by density in specific gravity tables. After the cleaning step, the husk is removed by passing rice kernels through two spinning rubber rolls at different speeds. Brown rice is obtained after dehulling; this kernel may be eaten as is, milled into white rice, or processed for obtaining different products and byproducts. The brown color of this kernel is caused by the presence of bran layers, which are rich in minerals and vitamins.

Therefore milling of paddy rice produces milled rice, broken rice, rice bran, and hulls and husks. Numerous products with added value have been developed from rice, such as convenient rice forms like rice flour, puffed and crisped rice, breakfast cereals, and snacks (Barber and Benedito 1970; Nguyen and Tran 2000; Wilkinson and Champagne 2004).

22.3 PARBOILED RICE

Paddy rice is the principal raw material for the production of parboiled rice, which is rice that has been boiled in the husk. This rice is obtained by a process of soaking at a temperature below the gelatinization temperature of the starch, steaming with or without pressure for several minutes, and cooling and slowly drying to minimize the formation of cracks. The parboiling process changes the nutrient profile, permitting the retention of many of the natural vitamins and minerals in the kernels that diffuse from bran to endosperm, without allowing the diffusion of fat and proteins. The practice of parboiling is more than 2000 years old, and it appears to have started in India. Most parboiled rice is milled into "white" rice, although the electric power consumption and broken kernel rate are higher than those for milling untreated brown rice (Itoh and Kawamura 1991). The resulting rice is slightly yellowish, although its color intensity decreases after cooking. There are numerous patents dealing with processing parameters for obtaining parboiled rice with a high degree of whiteness, good palatability, taste, and appearance (Lay William 1972; Satake 1972; Hunnell 1974; Hayashi 1982; Itoh and Kawamura 1985; Gebhardt and Lehrack 1993; Islam and others 2002a,b).

The parboiling process shifts the nutrients, mainly thiamine, from the bran into the grain, and the starch is gelatinized (Nicholls 1947; Simpson 1951). Drying of the gelatinized starch in the grain leads to clear and harder endosperm, more resistant to breaking during milling. Parboiled rice requires longer cooking because the gelatinized starch is more resistant to water absorption. Parboiled "white" rice is nutritionally similar to brown rice, and the cooked rice is firmer and less sticky. Parboiled rice is used as a raw material for the subsequent production of canned rice, and other processed rice products, due to its stability to overcooking. Puffed-rice products are also obtained from parboiled rice subject to pressure steaming, due to its higher volume expansion.

22.4 BROWN RICE AND GERMINATED BROWN RICE

Brown rice is obtained after dehulling, owing its brown colour to the presence of bran layers, which are rich in minerals and vitamins (Fig. 22.3). Brown rice contains more nutritional components than ordinary milled rice grains, such as dietary fiber, phytic acids, E and B vitamins, and γ-aminobutyric acid (GABA). All these compounds are present in the bran layers and germ that are removed during polishing or milling (Champagne and others 1991, 1995, 2004). Despite the nutritional benefits linked to the

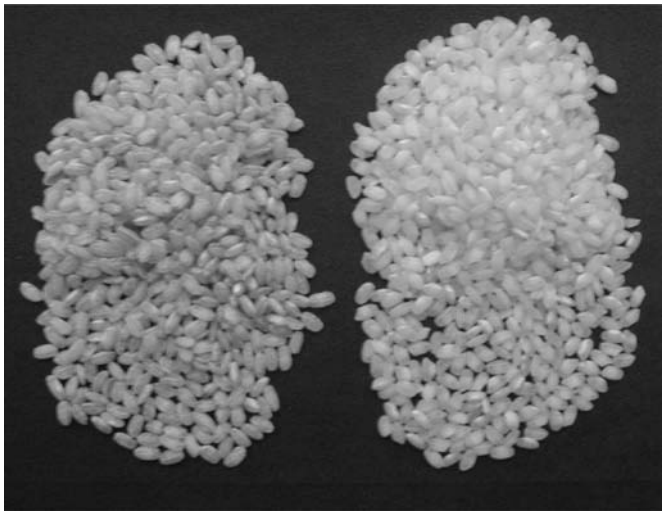

Figure 22.3 *Brown rice (left) and milled our polished rice (right). (photo by Cristina M. Rosell).*

consumption of brown rice, it is not considered suitable for table rice because it has to be cooked in a pressure rice cooker or for a longer cooking time; moreover, it has a dark appearance and hard texture. In addition, when the husk is removed from rice, the bran layer starts going rancid, contributing to the bitter taste of brown rice. Therefore, brown rice is mainly used as a medium for fermentation, or in materials for food processing.

The use of germination in grains started some decades ago and was applied to wheat and soybean (Finney 1978; Tkachuk 1979). Germinated brown rice arose following the policy of developing new value-added products from rice. In 1994, Saikusa and others found that GABA increased significantly when brown rice was soaked in water at 40°C for 8–24 h. An increase in GABA intake suppresses blood pressure, improves sleeplessness and the autonomic disorder associated with the menopausal or presenile period, or even suppressed liver damage (Tadashi and others 2000; Okada and others 2000; Jeon and others 2003). In Japan, germinated brown rice was launched to the market in 1995. Since then, germinated brown rice is increasing its popularity within the Japanesse population, and simultaneously numerous industries are emerging in Japan related to the production of germinated brown rice. During the last decade, 49 items related to germinated brown rice have been patented.

The basic procedure for obtaining pregerminated brown rice consists in the selection of good quality brown rice, which then is soaked for around 20 h at 30–40°C, changing the water a few times. This product is washed slightly before cooking, and is marketed either dry or wet, being with moisture contents of 15 and 30%, respectively. During the germination process, saccharification softens the endosperm and dormant enzymes are activated, increasing the amount of digestible compounds (Manna and others 1995). In addition, this process changes the mineral content, and results in an increase of GABA, free amino acids (Fig. 22.4), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitor (Kayahara and Tsukahara 2000; Ohisa and others 2003; Ohtsubo and others 2005). It can be cooked in an ordinary rice cooker, obtaining a soft product with easier chewiness. Germinated brown rice is also used as a raw material for processing many different

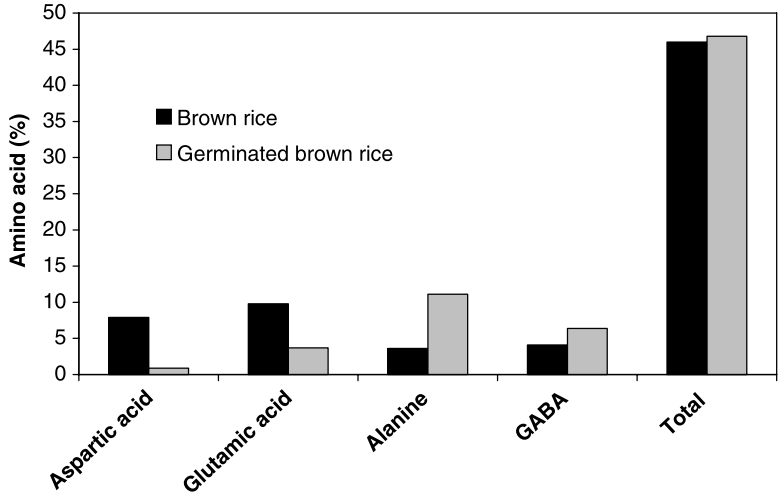

Figure 22.4 *Amino acid levels for brown rice and germinated brown rice.*

products, such as germinated brown rice balls, soup, bread, doughnuts, cookies, and rice burgers (Ito and Ishikawa 2004).

22.5 MILLED RICE

Milled rice is also known as white rice or polished rice (Fig. 22.3). Milled rice is obtained after removing the bran and germ from the brown rice. There are many machines and methods designed for milling rice, but often an abrasive system followed by frictional and polishing systems are used.

Rice milling can yield from 4 to 40% broken kernels depending on the incoming rice quality and the milling equipment. Broken kernels are removed from the whole kernels through indent graders in order to obtain high-quality table rice. Broken kernels can be further separated into various sizes according to their final use (brewing, screening, flour milling). Milled rice is usually consumed after boiling, but different techniques have been developed for the transformation of milled rice into a variety of tailored products.

Precooked rice is used for convenient rice-based products, ready to eat, where other ingredients are packed separately and mixed only after heating. These precooked rice products are packed in laminated plastic or aluminum-laminated plastic pouches and pasteurized at 120°C under pressure (Tani 1985). Steamed rices can be packed in aluminum-laminated plastic film pouches and only need to be warmed in hot water for 10–15 min. Steamed rices packed in plastic pouches, however, only need to be heated in a microwave oven for 1–2 min after puncturing of the bag. Quick-cooking rice requires significantly shorter cooking times (5 min) than the milled rice (15–25 min) (Juliano and Sakurai 1985; Luh 1991). Different technologies are suited for obtaining quick-cooking rice, like soak-boil-stem-dry methods, gelatinize-dry-puff methods, dry-heat methods, freeze-thaw methods, and freeze-drying (Roberts and others 1980; Jacops and Lin Yah 2000; Minier and others 2001). Parboiled rice can be converted to quick-cooking rice by reducing 1% of the pericarp in order to eliminate the outer water-impermeable layer.

Frozen cooked rice is packed in airtight plastic pouches and frozen in an instant in a sharp freezer. Freezing without dehydration is the most effective method for avoiding the retrogradation process. This convenient rice food is usually delivered to restaurants where they are heated in microwave ovens before being served to consumers.

Canned rice is a white product that has separate, noncohesive grains and contains a clear canning liquor. This product is consumed in the United States, Japan, the Philippines, and Taiwan. The grain is usually parboiled before canning to increase stability, and it also an acid pH (below 4.6) to reduce microbial contamination spoilage problems (Barber 1967a; Juliano and Hicks 1996).

22.6 ENRICHED OR FORTIFIED RICE

From the nutritional point of view, rice is considered an important source of energy, providing 26% of the total energy intake in developing countries and only 4% of the total energy intake in developed countries. In developing countries, rice supplies 20% of the dietary protein intake, but because of its incomplete amino-acid profile and the limited amount of micronutrients in the milled rice, there is a great problem of malnutrition where rice is a staple food. Today, different techniques for rice fortification have been developed in order to add essential vitamins and minerals to the grain (Barber 1967b; Rosell 2004; Hoffpauer and Wright 1994).

Food fortification is one way of solving nutrient deficiencies in developing countries, and so far the implementation of those programs has been very successful for correcting nutritional deficiencies within a very short period. In the developing countries, it should be taken into account that the food selected as a vehicle for the nutrient should be stable and consumed by the population at risk, and the amount of nutrient added should be sufficient to correct the possible deficiency. In general, all the rice-consuming countries have a vitamin A deficiency, which is associated with corneal lesions that can lead to partial or total blindness, and also with reduced resistance to infectious diseases, and in consequence an increased morbidity and mortality. Another deficiency associated with rice-consuming countries causes nutritional anemia due to iron deficiency, which has been linked to reduced resistance to infections and also effects on cognitive development and physiological functions in children, and in severe cases of deficiency causing maternal deaths. Iodine constitutes the third major deficiency in rice-consuming countries. This mineral is necessary for correct fetal development and also for normal physical and mental activities in adults.

There are different methods of rice fortification, one of which is the parboiling process (Nunes and others 1991). Other methods currently used are the so-called powder enrichment and grain enrichment. In powder enrichment, a preblended powder mixture of vitamins and minerals is added to the rice. For "white" parboiled rice, the blend is added immediately after milling, because the heat and moisture content of the grain surface at that moment facilitates adherence of the powder mixture. However, the nutrients supplemented in this way can be washed off if the grains are rinsed before cooking.

The grain enrichment method involves the addition of a powdered mixture of vitamins and minerals and a subsequent coating of the grain with a water-insoluble substance. Usually, grains enriched by this method contain a high nutrient concentration and are mixed in a 0.5% proportion with normal milled rice, to finally obtain an enriched

product that meets the required standard levels. Different variations of these methods have been developed in recent years; for instance, the powdered mixture can be sprayed onto the rice kernels and then fixed by the application of a water-insoluble coating such as ethanol, isopropanol, palmitic acid, or cellulose derivatives. This method has been successfully used for enriching rice with niacin, thiamin, pyridoxin, vitamin A, vitamin E, folic acid, iron, and zinc by adding alternative layers of nutrients and coatings. The most recent method consists in the development of fortified simulated rice, obtained by extrusion of rice flour in the presence of vitamins and minerals to a rice kernel shape. The fortified simulated grains are then mixed with normal milled rice. The disadvantage of this method is that the consistency of the resulting grains after cooking is different from the natural product.

22.7 EXPANDED RICE PRODUCTS AND SNACKS

Dry rice breakfast cereals include rice flakes, oven, gun, or extruder-puffed rice, shredded-rice cereals, and multigrain cereals. These products are prepared by pressure-cooking in the presence of sugar, salt, flavorings, and sufficient water. Rice flakes are prepared in a similar way to wheat and corn flakes. Rice is cooked and coated with nutritious ingredients (skimmed milk, wheat germ, wheat gluten, and so on) and is then partially dried, tempered, and passed through flaking rolls before toasting in an oven (Wilkinson and Champagne 2004). In 1951, Roberts and others presented the use of parboiled rice for the production of expanded rice products.

Rice snacks include granola, breakfast, and energy bars containing rice in their formulation (Juliano and Hicks 1996). Some snacks are designed as functional foods, with health contribution claims like cholesterol reduction. An significant number of these products have been launched targeted at children, women, and other specific groups.

22.8 BROKEN RICE

Rice kernel can be cracked in the field or during the drying or milling process. Often these cracks induce the breaking of the kernel, generating broken rice, which is removed during the milling process because it tends to get mushy during cooking, decreasing the quality of the table rice. The different sizes of broken kernels, from the largest to the smallest, are called brewing, screening, and flour milling. In some countries, broken rice is sold as it is, but at a lower price than the milled rice. Broken rice is also utilized for in the production of beer, high-fructose syrup, flour and high-protein flour, starch, maltodextrins, glucose syrup, feed for livestock, spirits, and distilled liquors.

Broken rice kernels of rice can be ground into flour using three different methods (Yen 2004). Wet grinding consists of soaking the broken kernels in water, and after draining they are ground in the presence of water, in order to reduce the amount of damaged starch. The excess water is removed by drying and the flour is again ground, yielding the wet rice flour. This product is used in the production of different Asian specialities like Japanese cake, Taiwanese cake, Indian fermented foods, and so on. Wet milling in the presence of 0.3–0.5% NaOH is used for the production of rice starch and rice maltodextrins and syrups. Semidry grinding also involves soaking, draining, and grinding without using any excess water. The semidry ground flour has similar applications to

the wet rice flour. Dry grinding is also possible; in this case broken kernels are directly ground to different sizes. Dry rice flour is used for baking, baby foods, extrusion-cooked products, and in high-protein flour.

22.9 RICE FLOUR

Rice flour is extensively used for the production of infant food formulas due to its digestibility and hypoallergenic properties. A partial acid or enzymatic (starch-hydrolyzing enzymes) hydrolysis of the rice flour is applied in order to increase the amount of free sugars, contributing to its sweet taste and consistency (Cantoni 1967).

The use of rice flour in baking has been progressively increasing, motivated by its usefulness as a wheat substitute in the dietary habits of wheat-intolerant patients. Celiac disease, first considered to be a gastrointestinal disease, is a gluten-sensitive enteropathy with genetic, immunologic, and environmental bases. Peptides released during gluten digestion are responsible for the intolerance in genetically predisposed individuals. Therefore, the only treatment is to keep their diet as gluten-free as possible. The unique cereals considered gluten-free safe are rice and corn, although rice flour is the most suitable cereal grain flour for the production of gluten-free products due to its bland taste, white color, digestibility and hypoallergenic properties (Neumann and Bruemmer 1997). In addition, other attributes such as its low protein and sodium content, low levels of prolamins and the presence of easily digested carbohydrates make the rice the selected cereal for patients suffering from celiac disease.

However, in spite of the numerous advantages of rice flour, rice proteins, compared with other plant proteins, have relatively poor functional properties for food processing. Rice proteins are extremely insoluble due to their hydrophobic nature and are unable to form the viscoelastic dough necessary to hold the carbon dioxide that is produced during proofing of yeast-leavened bread-like products. The low content of prolamins in rice flour prevents the formation of a protein network when rice flour is kneaded with water. As a consequence, the carbon dioxide produced during fermentation cannot be retained, yielding a product with a low specific volume that does not resemble the soft and open structure of wheat bread (He and Hoseney 1991).

In order to overcome this problem, different structuring agents have been added to rice bread formulation (Nishita and others 1976). Xanthan gum and carboxymethylcellulose (CMC) have been used as gluten substitutes for preparing gluten-free bread (Kulp and others 1974). Xanthan gum has been used as a gluten replacer in the preparation of cornstarch bread, yielding baked bread with a good specific volume, but a coarse crumb texture and lack of flavor (Christianson and others 1974). Among the cellulose derivatives, hydroxypropylmethylcellulose (HPMC) seems to be the best gluten substitute in rice bread formulas due to its gas retention capacity and its properties as a crumb-structuring agent (Nishita and others 1976; Ylimaki and others 1988; Gujral and others 2003a). With the addition of HPMC, it is possible to obtain rice flour dough having a similar consistency and rheological properties to those of wheat dough (Sivaramakrishnan and others 2004). Other gums such as locust bean gum, guar gum, carrageenan, xanthan gum, and agar have been tested as gluten replacers in rice flour bread, but the addition of HPMC or the combination of HPMC and carboxymethylcellulose (CMC) has resulted in the highest specific loaf volume due to their ability to trap fermented gases (Kang and others 1997; Cato and others 2004).

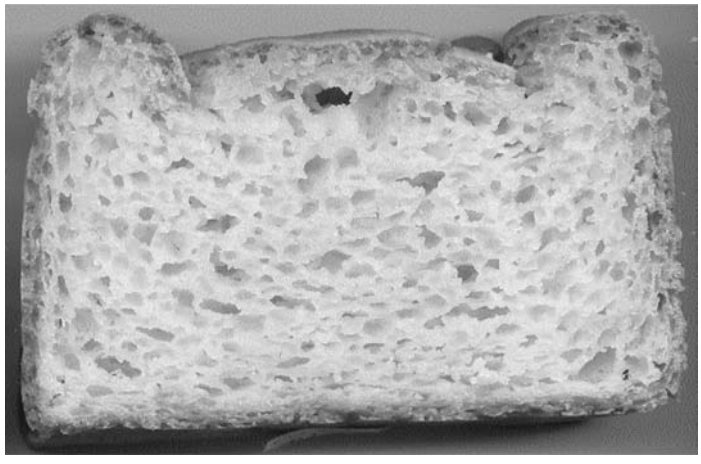

Figure 22.5 *Rice-based breed.*

Some enzymes, such as cyclodextrin glycosyl transferase, glucose oxidase, or transglutaminase, have been added to the rice bread formulation for obtaining a further improvement on the technological quality of the rice-based bread (Gujral and others 2003a,b; Gujral and Rosell 2004a,b). A chemical modification of the rice flour has also been proposed for improving its baking functionality (Nabeshima and El-Dash 2004). All the different approaches allow rice-based bread to be obtained with similar texture characteristics to wheat bread (Fig. 22.5).

Some bread specialties have been adapted for obtaining gluten-free products targeted at people suffering from gluten intolerance. This is the case for chapati, unleavened bread made from wholewheat in India. The use of different hydrocolloids (HPMC, guar gum, xanthan gum, locust bean gum) and α-amylase in the formulation of rice-flour chapati has improved the texture by maintaining its extensibility during storage (Gujral and others 2004).

A different approach for obtaining gluten-free bread concerns the use of rice flour blended with other flours and different starches (Gallagher and others 2004). Complex formulations including corn starch, brown rice, soy, and buckwheat flour have been proposed for obtaining gluten-free bread (Moore and others 2004). With these recipes, the breads were brittle after two days of storage, although that effect was reduced in the presence of dairy products like skimmed milk powder, which yielded a network-like structure resembling the gluten network in wheat bread crumb. A combination of rice flour (45%) with corn (35%) and cassava (20%) starches gave also a good gluten-free bread with uniform and well-distributed cells over the crumb, and with a pleasant flavor and appearance (Lopez and others 2004). Gluten-free breads of good quality were also obtained by lowering the amount of rice flour (17.2%) and using corn starch (74.2%) and cassava starch (8.6%) (Sanchez and others 2002). Soy protein is frequently added to gluten-free bread formulations because it improves the bread texture (Sanchez and others 2002; Sodchit and others 2003). Blends of buckwheat and rice flour in the presence of hydrogenated vegetable fat also gave gluten-free breads with good sensory acceptance (Moreira and others 2004).

In breadmaking of wheat breads, brown-rice brown flour has been used as an ingredient, although a reduction in the specific bread volume was observed (Watanabe and others

2004). Pregerminated brown rice is another alternative as bread ingredient. The substitution of pregerminated brown rice flour for wheat flour improves the bread quality and retards the staleness of bread staling (Watanabe and others 2004).

Rice cakes and crackers are increasing in popularity due to their tendency to be associated with healthy products. Rice is often used in cracker and chip production because it contributes to moisture control, texture and flow in the extruders (Wilkinson and Champagne 2004). Japanese specialties of rice cakes and crackers include senbei and arare (Nguyen and Tran 2000). In rice-cake production, the gluten network is not developed; thus it is possible to make cakes with starchy raw materials like starch from different cereals. Therefore, rice flour can be employed in the production of cakes as the main ingredient or blended with wheat flour (Bean and others 1983; Rosell and Gomez 2006). The quality of the rice cakes can also be improved by adding different proteins, like skimmed milk powder or egg proteins (Mohamed and others 1995; Mohamed and Hamid 1998). Rice flour has been used in the production of donuts. The use of rice flour led to less dough consistency and harder products with less moisture content and higher oil absorption (Shih and others 2001), but this problem could be overcome with the use of pregelatinized rice flour or acetylated rice starch (Shih and Daigle 2002). The use of rice flour has also been described in the production of cookies (Przybyla and Luh 1977; Schober and others 2003).

22.10 HULLS AND HUSK APPLICATIONS

Rice hulls and husks account for about 20% of grain weight. They are used as an energy source in some countries (Kapur and others 1996), whereas in others, they are considered as waste and used for animal feed (Gonzalez and others 1986). Different technologies have been developed for using hulls and husks, but always the application feasibility depends on the process economy. Rice husk has been used for obtaining rice husk ash or silica ash after subjecting the rice husk to a burning process. Silica ash, composed mainly of silica, is used as filler in a variety of polymeric composites employed for the production of such materials as tiles and ceramics, or even road-building materials (James and Rao 1992; Chaudhary and others 2004). Other uses include bedding material for poultry, horses, or ducks, incubation material for duck eggs, or seedbed media for vegetable production.

22.11 BRAN APPLICATIONS

Rice bran accounts for 5–8% of the paddy rice weight, and comprises germ, pericarp, aleurone cells, and seed coat. Rice bran is a byproduct of rice milling with a high content of proteins, fat, and other nutraceutical compounds. However, the presence of the enzyme lipase, which causes a rapid deterioration of lipids, obliges to inactivate the enzyme for bran stabilization. Heat treatment, low-temperature storage, chemical treatment, control of relative humidity, and simultaneous milling and extraction are different economically doubtful alternatives used in stabilizing the bran (Anon. 1985). As a consequence, rice bran has been widely used as livestock feed, boiler fuel, and for obtaining rice bran oil (Tortosa and Benedito de Barber 1978). Rice bran has been used in food as full-fat rice bran, defatted rice bran, or in the form of protein concentrates and rice bran oil. Protein concentrates from rice bran contain from 19.4 to 76.1% protein content depending

on the extraction method (Barber 1971; Barber and Maquieira 1977; Barber and others 1981; Shih 2003), and they have been incorporated into bread, beverages, confections, and weaning foods (Prakash 1996).

Rice bran contains 15–25% lipids, and the oil extracted from rice bran is unique among edible oils due to its high content of commercial and nutritional phytoceuticals such as oryzanol, lecithin, tocopherols, tocotrienols, and sterols. However, most of these compounds are removed from the rice bran oil during the refining process. Different methods of extraction have been proposed in order to recover all these compounds because of their applications in nutraceutical, pharmaceutical, and cosmoceutical preparations (Patel and Naik 2004; Danielski and others 2005). Lately, oil from rice bran has received increased attention as a possible source of renewable diesel fuel or biodiesel (Ju and Vali 2005).

Rice bran also has a direct use as a medium for screening *Saccharomyces* species or for the production of protease and milk-clotting enzymes.

Rice is one of the most consumed cereals, and its main way of consumption is as milled rice. However, the consumption decrease experienced in rice-based products over the last decades has stimulated the development of a number of different value-added rice products. Apart from the use of rice as table rice, there is a high consumption in beer production, baby foods, breakfast cereals, snacks, confections, desserts, and also in bakery products. The increased use of rice for food processing has been the result of an increasing consumer demand for more healthy products, more convenient products, and also a growing interest in a variety of ethnic products. In addition, husks, hulls, and bran are used as energy sources, fillers of polymeric composite, and raw materials for the production of nutraceuticals and protein concentrates.

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23

Asian (Oriental) Noodles and Their Manufacture

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23.1 Introduction 539
23.2 Classification of Asian (Oriental) Noodles 541
23.3 Wheat-Based Asian Noodles: Characteristics and Manufacturing 542
23.3.1 Wheat-Salted Asian Noodles 543
23.3.2 Yellow-Alkaline Wheat-Based Asian Noodles 545
23.3.3 Egg Noodles 546
23.3.4 Instant Products 549
23.3.5 Savory Noodles 552
23.4 Brownish Buckwheat Asian Noodles 554
23.5 Rice-Based Asian Noodles 556
23.6 Starch-Based Asian Noodles 559
23.7 Yam Noodle 561
23.8 Variables Affecting Product Quality of Asian (Oriental) Noodles 561
23.9 Conclusions 562
References 563

23.1 INTRODUCTION

The consumption of noodles has a long history. Westerners have claimed that noodles originated from Italy. However, the Chinese claim that noodles orginated in China and were brought back to European countries on Marco Polo's return trip to his home

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country. With the recent archeological discovery of a bowl of noodles made from a dough of broomcorn and foxtail millet dating back to the Late Neolithic era in the Qijia culture in Western China, it appears that the Chinese are winning this battle.

The term "mien (mian, or mein)" in the Chinese language is used to describe all noodle-type products (with a few exceptions in shape) made from common wheat flour as the main structural ingredient. It is wheat-based, as shown by the traditional Chinese character for noodles, which has "mia (wheat)" on one side and "mien (sheet)" on the other side of its character structure. For rice noodles, the Chinese character is written with "mi (rice)" on one side of the character structure and the other side indicates "fen (separate)", indicating that it originated from starch isolated from rice. Similar products made from mung beans and other ingredients are all grouped under the term "fen (starch-based products)", for example, "mi fen (rice noodle)", "tung fen (mung bean threads)", and "ho fen (oily rice strips)". It is assumed that "mien" and "fen" were spread from China to its neighboring countries, because the terms for noodle-like products in these countries are very similar in their sounds: for example, "men" (Japanese wheat-based noodle), "mie" (Indonesian noodles), "mee" (Malaysian noodles), "Pho" (Vietnamese rice-based noodle). Noodles made in China are traditionally continuous instead of short pieces. It is also customary to serve (continuous) noodles at birthday parties, symbolizing longevity.

The terms "noodles" and "pasta" are often used interchangeably by consumers. However, they are technically different. Western-style pasta uses durum wheat flour as the main structural ingredient, but Asian noodles are usually made from regular wheat flour. In the United States, there is a legal term (standard of identity) for "noodle", which must contain a certain proportion of egg solids. However, with an increase in the number of Asian immigrants in the United States, and their production and consumption of noodle-like products from their native countries (with some of them now produced in the United States), FDA finally accepted the term "Asian (Oriental) noodles" to describe a group of products that are similar to the American noodle, but differ in their composition and appearance with the mandate that a decsriptive or qualifying term must appear before "noodles": for example, Chinese noodles or rice noodles. Thus, in the United States, "Asian (oriental) noodles" are mostly noodle-like products produced mainly in Eastern, Southeastern, or Pacific Asian countries using common wheat flour, rice (or rice flour), or other starch materials as the main structural ingredient. Some of these products are now produced in the United States. However, there is no standard of identity for Asian noodles in the United States. This situation may change in the future and certainly differs in other countries.

Wide variations exist among the various Asian noodles in size, appearance (color and shape), ingredients, chemical properties, and methods of manufacture. In this chapter, some of these variables are introduced briefly. It should be noted that only carbohydrate-based Asian noodles are presented in this chapter. Other so-called Asian noodles, such as seaweed noodles (cut-up seaweed strips) and tofu noodles (semidry tofu strips), are not included in this chapter.

Several excellent reviews on Asian (oriental) noodle production are available. Readers should consult these references for further information (Hoseney 1994; Huang 1996a,b; Kim 1996a,b; Nagao 1996; Udesky 1988; Corke and Bhattacharya 1999; Hatcher 2000; Hou 2001; Crobie and Ross 2004; Ross and Hatcher 2005; Lu and Nip 2006). Variables affecting the production of these Asian (oriental) noodles are also studied to some extent (see following sections for further information). There is no doubt that literature in Japanese, Korean, Chinese, and other Eastern Asian languages are also available, but they are not available at the time of this work.

23.2 CLASSIFICATION OF ASIAN (ORIENTAL) NOODLES

Asian noodles vary considerably in size, appearance (color and shape), ingredients, chemical properties, and methods of manufacturing. There are several ways to classify Asian noodles. One of the classifications is based on the major ingredients used in the manufacture of these noodles (Table 23.1). The major ingredients are wheat flour, buckwheat flour, rice flour, starch of various origins, and gum. Within the wheat flour category, the noodles may or may not contain egg solids, alkaline agents, and savory flavorings. Another classification is based on the changes of starch properties during the manufacturing process (Table 23.2). The starch may stay in the natural state, partially altered or completely gelatinized, producing products that are opaque, semitranslucent, and of a cellophane-type appearance, respectively. A third classification method is based on the appearance (thickness and/or width) of the noodles. This classification is designed to standardize wheat-based noodles in China and Japan (Table 23.3).

TABLE 23.1 Classification of Asian Noodles by Major Ingredients.

Base Ingredient	Name of Noodle	Ethnic Origin	Other Ingredient(s)
Wheat flour	Gook soo	Korean	
	Kal guk su	Korean	
	Kishimen	Japanese	
	Somen	Japanese	
	Hiyamugi	Japanese	
	Udon	Japanese	
	Miswa	Filipino	
	Pancit Canton	Filipino	Coconut oil, coloring
	Egg noodle	Chinese	Egg solids
	Mein/Main	Chinese	
Buckwheat	Cha soba	Japanese	
	Soba	Japanese	
	Naeng myun	Korean	Sweet potato flour optional
Rice flour	Ban pho	Vietnam	
	Bun	Vietnam	
	Mai fun	Japanese	
	Mei fun	Chinese	
	Ho fen	Chinese	
	Pancit bihon	Filipino	
	Chantaboon	Thai	
	Rice sticks	Various cultures	
Various starches			
Mung bean	Fen si (mungbean threads)	Chinese	
	Sai fun	Chinese	
	Bun tau	Vietnam	
Sweet potato	Dang myun	Korea	Corn starch optional
	Soo foon	Malaysian	
	Harusame	Japanese	Other starches
Corn/tapioca/potato	Starch noodles	Various cultures	
Devil's tong yam	Shirataki	Japanese	
	Ito konnyaku	Chinese/Japanese	

TABLE 23.2 Classifcaion of Asian Noodles by Their Starch Properties.

Starch Properties	Noodles
Raw	Most wheat noodles except instant noodles and other deep-fried noodles
Partially gelatinized	All rice noodles
Pregelatinized	Cellophane or starch noodles
Gelatinized	Instant noodles and other deep-fried noodles

TABLE 23.3 Classification of Wheat-Based Noodles in China and Japan.

Classification	Chinese Examples	Japanese Examples
Very thin noodles	Longxu noodles (China) Yinsi mien (silver threads) (Hong Kong)	Somen
Thin noodles	Xi mien (China, Hong Kong, Taiwan)	Hiya-mugi
Flat noodles	Yangchun mien (China)	Kishi-men
	Kuan (broad) mien (China, Taiwan)	Hira-men
Standard	Not available	Udon
Wide flat noodles	Dai mien (China)	Not available
Thick noodles	Ci mien (Shanghai, China) (similar to Japanese udon)	Not available

23.3 WHEAT-BASED ASIAN NOODLES: CHARACTERISTICS AND MANUFACTURING

Wheat-based Asian noodles can be subdivided into the following major categories:

- White-salted;
- Yellow-alkaline;
- Egg noodles;
- Instant (deep-fried or dried) products; or
- Savory.

Table 23.4 compares selected chemical properties of white-salted, yellow-alkaline, instant, and savory wheat-based Asian noodles.

TABLE 23.4 Comparison of Selected Chemical Properties of Various Types of Wheat-Based Asian Noodles.

Chemical Properties	White-salted	Yellow-alkaline	Instant	Savory
Protein in wheat flour, %	8—10	10–12	8–12	10–12
Water added, %	30–35	30–35	30–36	30–35
Salt added, %	2–3	Variable	Variable	Variable
Alkaline agent(s) added	No	Yes	Yes/no	Yes/no
pH	6.5–7	9–11	6.5–7 (regular-type) 9–11 (alkaline-type)	6.5–7 (regular-type) 9–11 (alkaline-type)
Oil added, %	None	None	15–21.5 (fried-type) 1.5–1.8 (dried-type)	Variable Variable

Source: Adapted from Lu and Nip (2006).

23.3.1 Wheat-Salted Asian Noodles

The ingredients for this type of Asian noodles are common wheat flour, salt, and water, with egg solids as an optional ingredient. They have a soft elastic texture and smooth surface. Figure 23.1 is a generalized scheme for the industrial manufacture of plain wheat-based noodles in China. Products in this group are manufactured in a similar manner. These noodles vary in width and appearance considerably, with Chinese *longxu* (dragon whiskers) and *yinsi* (silver threads) noodles, and Japanese *somen* being typical examples of the very thin type of noodles (Fig. 23.2). Dried Japanese *somen* is usually wrapped with a piece of paper as a single serving quantity. Chinese *xi mien* and dried Japanese *hayamugi* are typical examples of thin noodles (Fig. 23.2). Hayamugi is always wrapped with a small piece of paper together with colored/dyed noodle pieces in a single serving bundle. Japanese *udon* and Shanghai *cu mien* (thick noodles) are examples of thick noodles

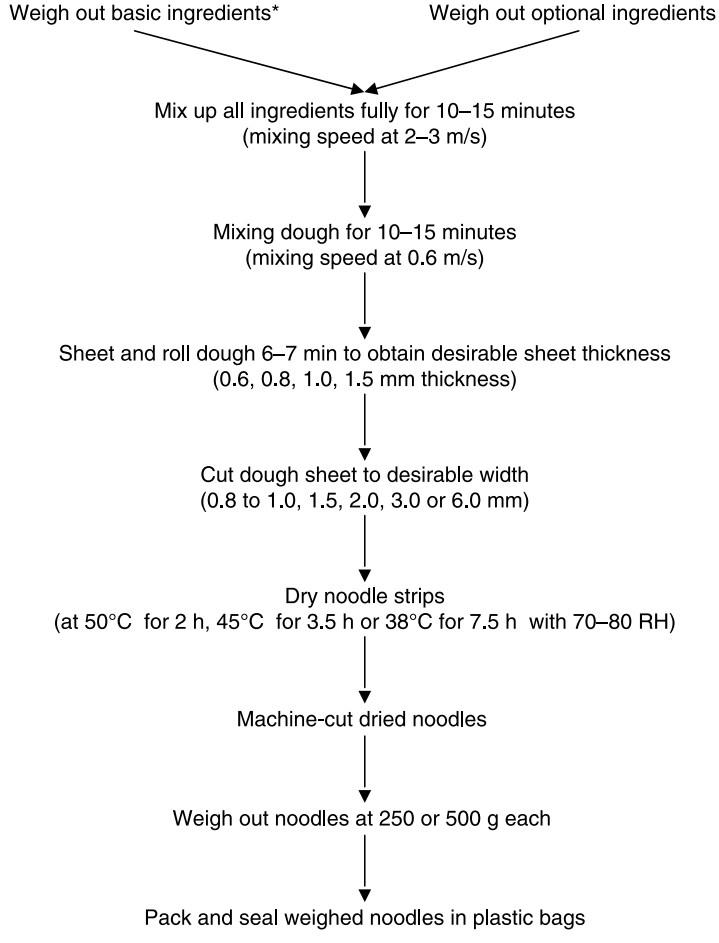

Figure 23.1 *Generalized scheme for the industrial manufacture of plain wheat-based noodles in China. *Basic ingredients: wheat flour (100 parts), water (25–32 parts dependent on gluten content of wheat flour at 30°C), salt (2–3 parts), alkaline agent (optional, 0.1 to 0.2% of flour weight). Adapted from Hou 2001, Huang 1996a,b.*

Figure 23.2 *Bundles of* somen *and* hayamugi *noodles.*

(Fig. 23.3). They are available in fresh and dried forms. Examples of flat noodles are dried Japanese *kirsimen* and *hiramen*, and Chinese *yang-chun mien* and *kuan mien* (wide noodles) (Fig. 23.3). *Dai mien* (China) and *cu mien* (China, Hong Kong, and Taiwan) are examples of wide flat noodles (Fig. 23.3).

At the household or restaurant level in China, plain noodles (such as knife-cut (pared) noodles, hand-cut noodles, cat's ear noodles, and hand-swung noodles (la mien)) can also be manufactured manually in small quantites. Although these noodles are made by hand, they have become delicacies in some areas as they are not easily produced and require some skills. They are often sold as fresh noodles, and cooked as soon as they are made. Figures 23.4 and 23.5 present the generalized schemes for the manufacture of pared, and hand-cut and cat's ear noodles, respectively. Manufacturing of hand-swung noodles

Figure 23.3 *Examples of thick noodles.*

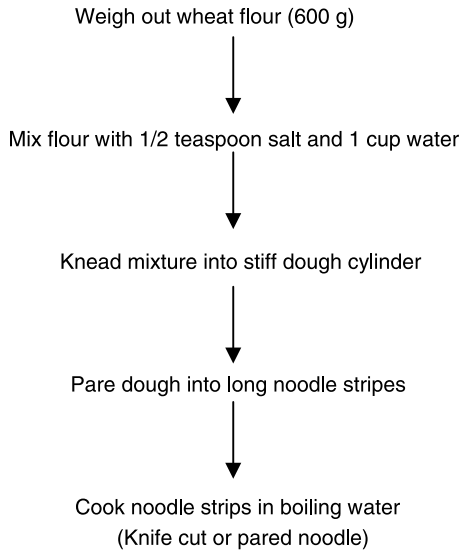

Figure 23.4 *Generalized scheme for the manufacture of pared noodles.*

or *La mien* is considered an art (Fig. 23.6), and this technique has been improved in Taiwan by applying simple devices for the stretching process instead of hand stretching/swinging (Figs 23.7 and 23.8).

In recent years, precooked, ready-to-eat *udon* in sterilized retortable pouches has been available. The cooked *udon* is sealed with water in the containers with lactic acid or sodium benzoate as preservatives, and sterilized for long-term storage.

23.3.2 Yellow-Alkaline Wheat-Based Asian Noodles

The basic ingredients for this type of noodles are common wheat flour, water, alkaline agents(s) (sodium and/or potassium carbonate), salt (optional), egg solids (optional), and yellow coloring (optional). Typical examples are Cantonese-type noodles with or without egg solids, Taiwanese-type noodles (Fig. 23.9), and *saimen* (commonly available in Hawaii). Figures 23.10 and 23.11 show generalized manufacturing processes for the production of Hong Kong or Cantonese-style alkaline noodles, and Taiwanese alkaline wheat noodles or *Yi-mien*, respectively. Addition of alkaline salts to the formulation not only alters the pH and color, but also improves the water absorption properties of the final product. It also improves the texture of the cooked product, making it chewier and with less tendency to soften and paste after cooking. The flavor of the cooked product is typical of an alkaline odor and influences its acceptance to consumers. For example, the majority of consumers in Hong Kong are used to this type of Asian (oriental) noodles. However, for most consumers in central and northern China, their preference is the common white-salted noodles. In some cases, a yellow coloring is added.

Frozen precooked *saimen* (an alkaline noodle) has also been available for many years in Hawaii. The *saimen* is precooked in water, or steamed, cooked, cooled, packaged, and sealed in plastic bags before freezing. The shelf-life of this product is good.

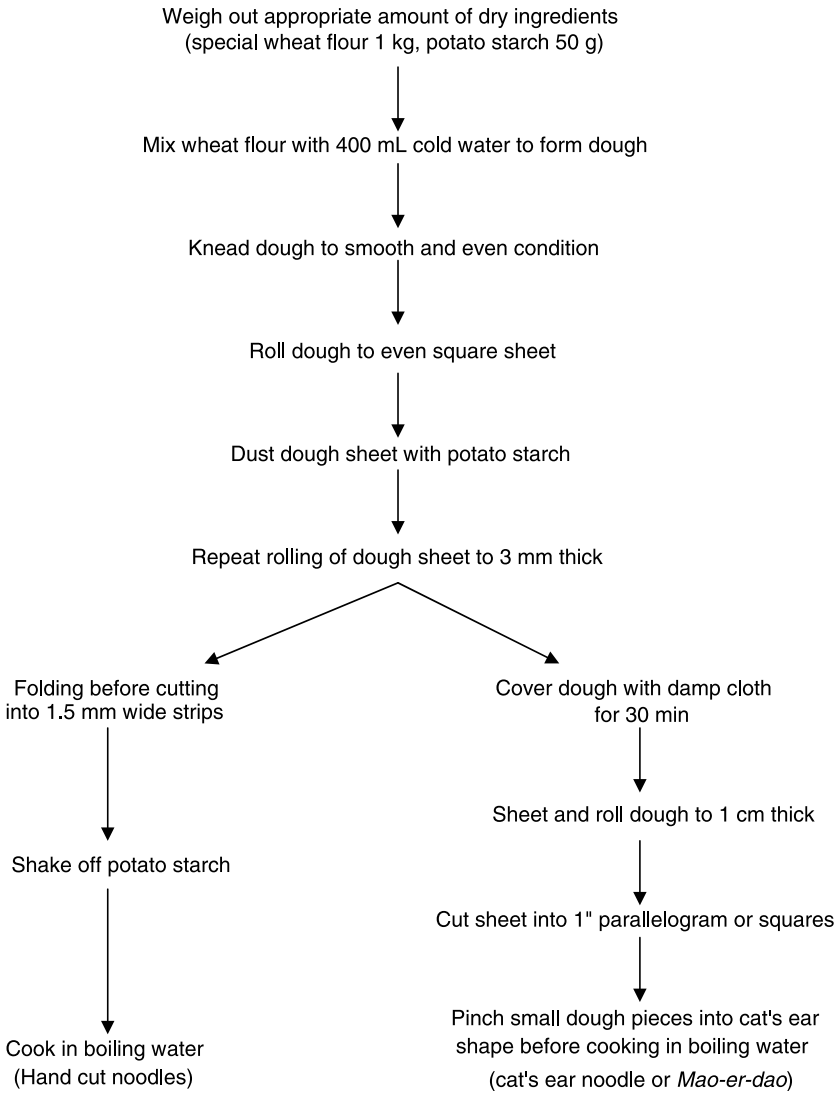

Figure 23.5 *Generalized scheme for the manufacture of hand-cut and cat's ear noodles. *1 kg of medium strength wheat flour without 10% protein, 20 g salt. **Dissolve 20 g salt in 500 ml water, water temperature at 25°C in summer and 35°C in winter. ***Dissolve 10 g sodium carbonate in 50 ml water. Adapted from Corke and Bhattacharya 1999.*

23.3.3 Egg Noodles

The use of egg soilds (whole, white, or yolk) in the manufacture of wheat-based Asian noodles has been practiced for many decades in Hong Kong and parts of China. The manufacture of whole-egg noodles (Fig. 23.12) and their consumption are fairly common practices in Hong Kong. It should be noted that egg noodles made in Hong Kong are usually bundled into an oblong or round shape (Figs 23.3 and 23.12, respectively). In some parts of China, it is common practice to add egg white and egg yolk

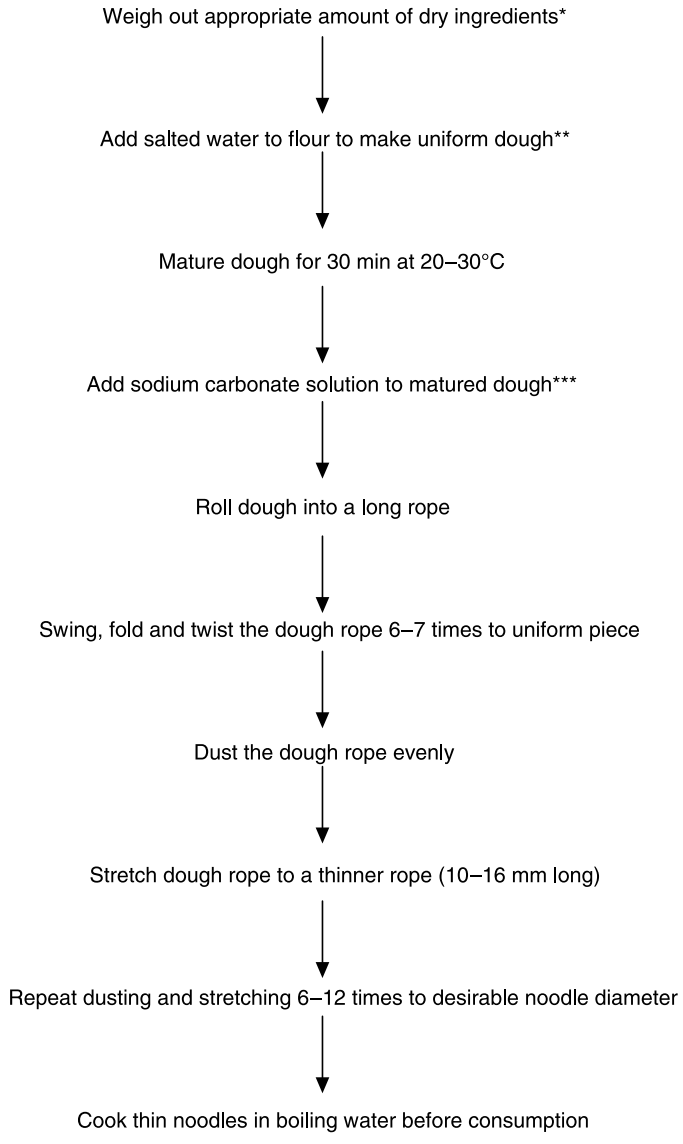

Figure 23.6 *Scheme for the manufacture of hand-swung noodles. * 1 kg of medium strength wheat flour without 10% protein, 20 g salt. ** Dissolve 20 g salt in 500 ml water, water temperature at 25°C in summer and 35°C in winter. *** Dissolve 10 g sodium carbonate in 50 ml water. Adapted from Corke and Bhattacharya 1999.*

separately to the wheat flour in the manufacture of two different wheat-based noodles (one white and the other yellow) and these two different kinds of noodles are used together in the preparation of a special bowl of noodles having two colors. The manufacture of egg noodles follows the basic manufacturing practice for dry wheat-based Asian noodles in China (Fig. 23.1). The addition of egg solids contributes to the color and textural properties of the final products. Whole-egg noodles have a rich flavor,

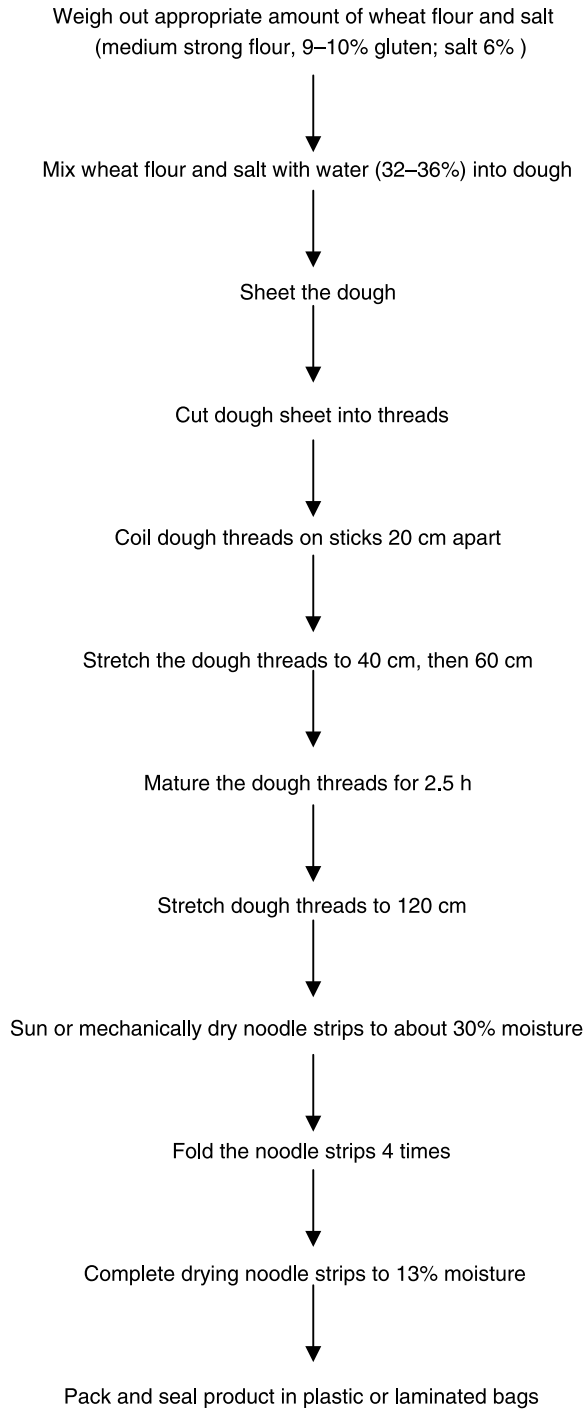

Figure 23.7 *Scheme for the manufacture of noodles using simple devices for the stretching process.*

Figure 23.8 *Simple stretching system for* La miern.

golden yellowish color, and firm and chewy texture. They stay fairly separate after cooking, especially after rinsing with cold water. The addition of egg white provides additional chewy texture and a whiter appearance, and addition of egg yolk contributes to the rich yellow color and a slightly softer texture because of the presence of lipids and lecithin in the egg yolk.

23.3.4 Instant Products

The majority of this type of products has been cooked either by deep-frying or steam-cooking and then dried following the procedure in the manufacture of regular wheat-based noodles. The starch has been gelatinized and the products can be consumed directly if

Figure 23.9 *Examples of yellow-alkaline wheat-based Asian noodles.*

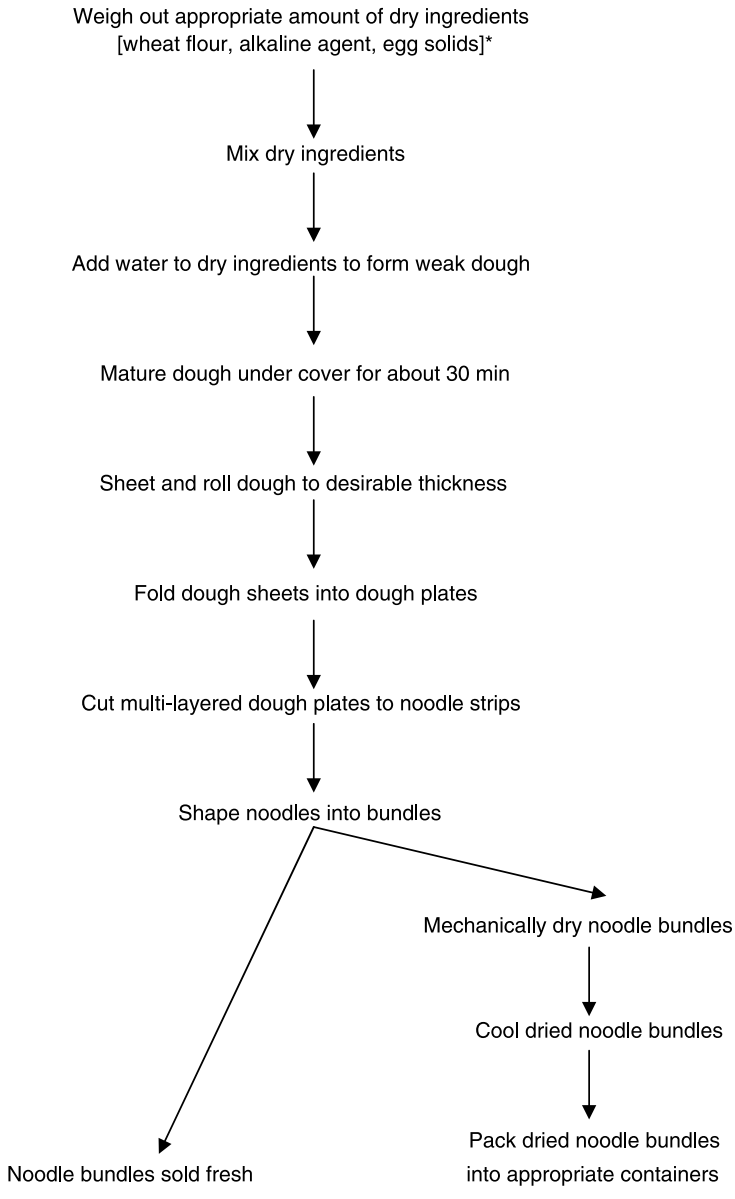

Figure 23.10 *Generalized scheme for the manufacture of Hong Kong or Cantonese-type alkaline noodles. *Alkaline agent can be sodium or potassium carbonate and egg powder can be whole egg, egg yolk or egg white. Adapted from Lu and Nip 2006.*

necessary or with minimal rehydration time in hot or boiling water. This is in contrast to the other types of Asian noodles, which require boiling in water to gelatinize the starch before consumption. Typical examples are instant noodles (such as *ramen*), and Cantonese *E-min* (deep-fried noodles) (Fig. 23.13). The American fried chow mein is a similar product. These noodles do not tend to leach out their starch as quickly as other

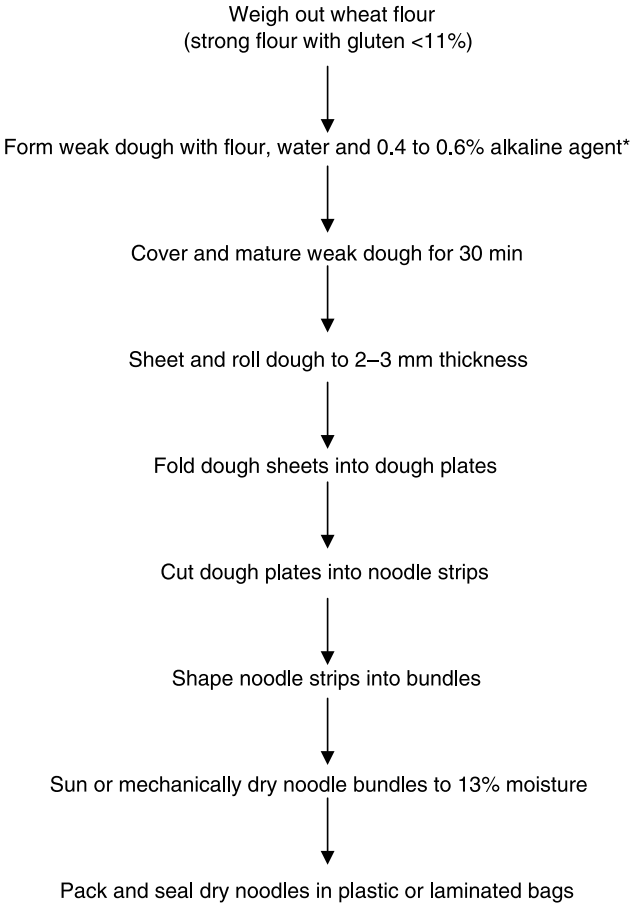

Figure 23.11 *Generalized scheme for the manufacture of Taiwanese alkaline wheat noodles. * Sodium and/or potassium carbonate. Adapted from Lu and Nip 2006.*

wheat-based noodles and have a soft but elastic texture. Their manufacturing processes are outlined in Figures 23.14 and 23.15. Traditional instant noodles are deep-fried, but now there are products that are not deep-fried, but just dried after steaming. This will satisfy consumers who do not want to have so much oil in the final products. Cantonese

Figure 23.12 *Chinese egg noodles.*

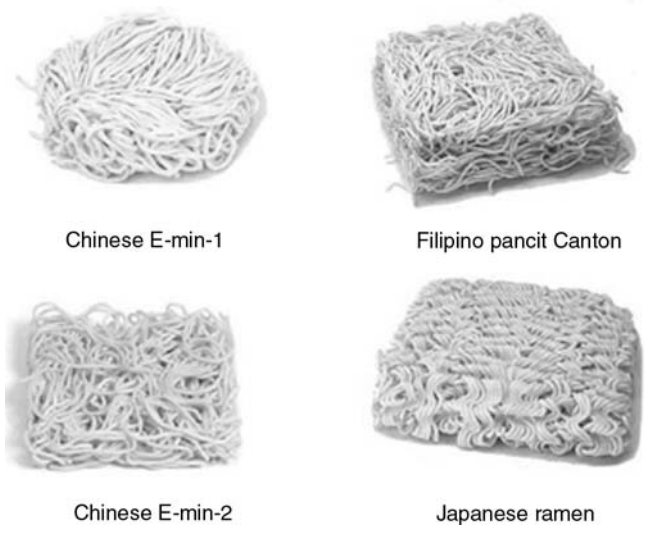

Figure 23.13 *Examples of instant noodles.*

E-min is always deep-fried, with an expansion in volume of three to four times, and it has a much longer history than instant noodles, which can be considered as an improvement of Cantonese *E-min*. It should be noted that ramen-type instant noodles (either deep-fried or dried) have a fairly compact appearance, but *E-min* has a puffed appearance (Fig. 23.13). Instant noodles are packed in cellophane pouches, or styrofoam/laminated paper cups/bowls. Recently, there are products available that contain sterilized pouches of cooked wheat-based noodles as part of the package.

23.3.5 Savory Noodles

This group of Asian noodles is considered as a specialty item. The savory flavorings may be shrimp eggs, egg yolk, egg white, whole egg, dried meat floss, tomato sauce, milk, soy milk, fish stock, chili powder, monosodium glutamate, butter, beef powder, prawn meat, L-lysine hydrochloride, chicken broth, spinach juice, or imitation flavors. They are manufactured mainly in China (including Hong Kong Special District). The manufacturing process is essentially the same as for regular dry wheat-based noodles (Fig. 23.1). The colors of these savory noodles vary according to the savory ingredients used.

The oily noodle is popular in Taiwan, southern China, and in some East Asian countries. It is unique in that oil is added to the cooked noodle to provide a special mouth-feel and it is served *al dente*. This oily, alkaline wheat-based *mien* is sometimes called *Hokkien*-type noodles. The procedures for preparing the raw *mien* are essentially the same as other alkaline wheat-based *mien*. They are then boiled once or twice, or steamed until they are completely cooked, before coating with oil and food coloring (optional). Figure 23.16 shows a generalized scheme of the manufacture of oily noodles. They are sold mainly as a fresh item and require minimal effort to prepare a dish for consumption.

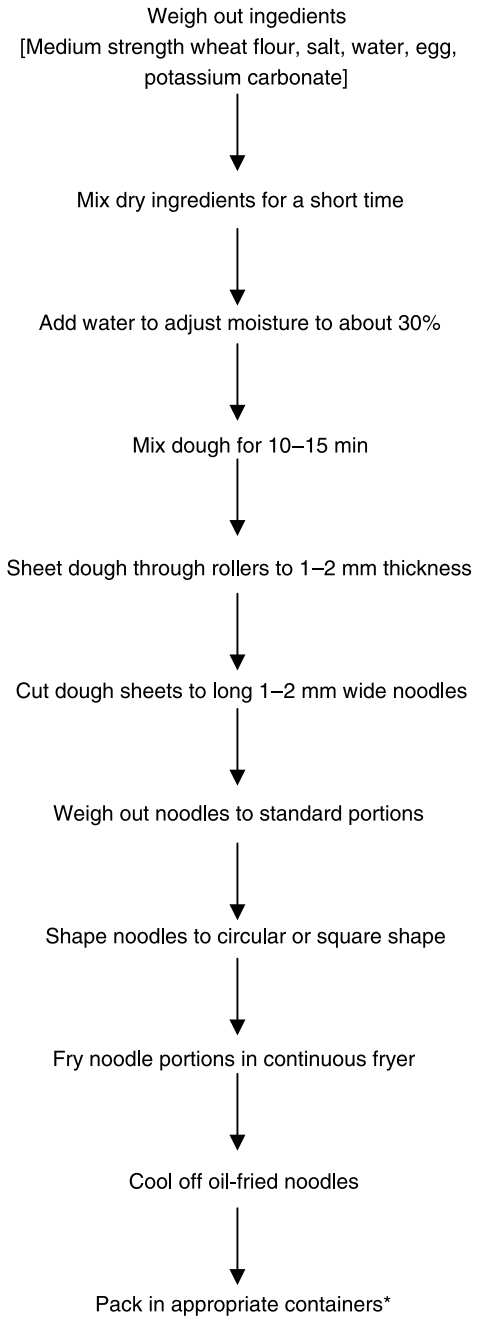

Figure 23.14 *Manufacturing process for instant noodles. * Fried noodles can be packed in cellophane bags, cardboard boxes, Styrofoam trays wrapped wrapped with cellophane, or vacuum packed in laminated pouches. Adapted from Lu and Nip 2006.*

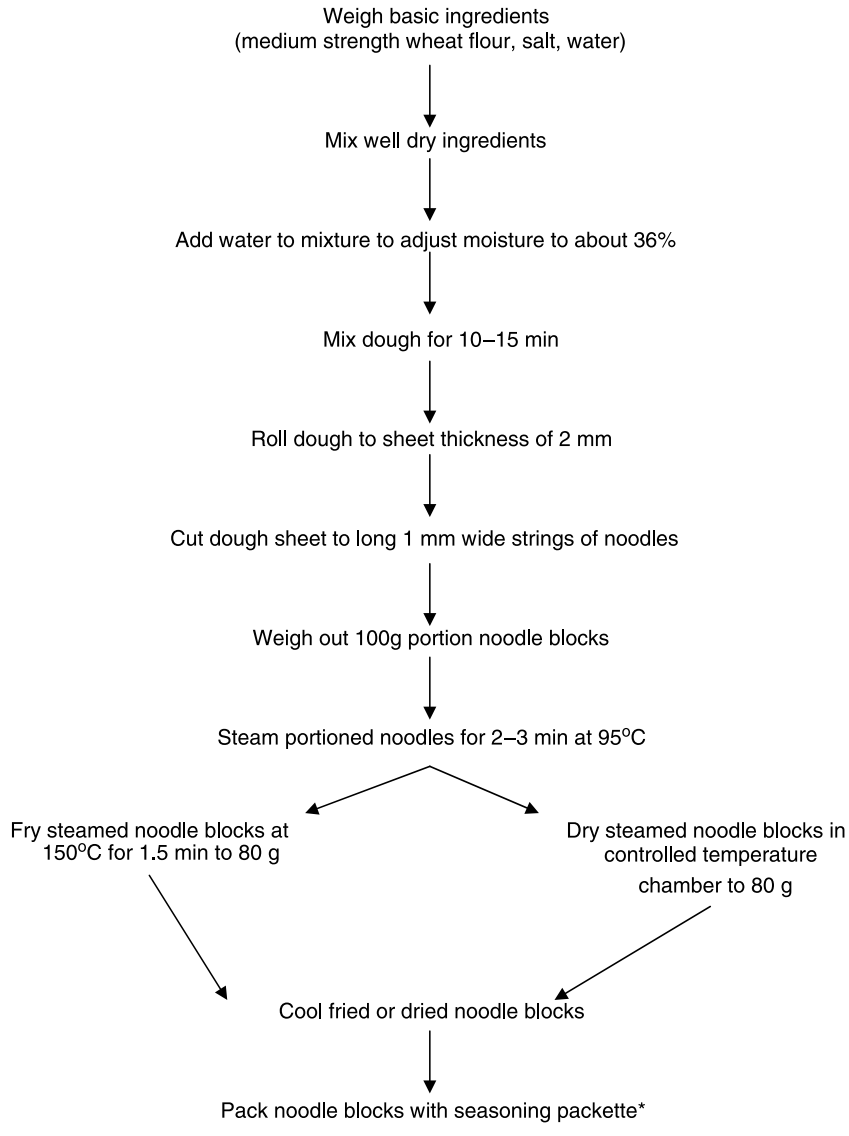

Figure 23.15 *Manufacturing process for instant noodles. *Packages may be plastic bags, or Styrofoam/plastic/laminated cups/bowls. Adapted from Lu and Nip 2006.*

23.4 BROWNISH BUCKWHEAT ASIAN NOODLES

The basic ingredients for manufacturing this type of Asian noodles are buckwheat flour, water, lime-wash or alkaline salts (optional), salt (optional), and yam flour (optional). Brownish buckwheat noodles made from 60–70 parts common buckwheat flour and 30–40 parts wheat flour have the following chemical properties:12–14% protein, 45–48% water, pH 6.5–7, and no salt or oil added. Addition of alkaline salts is optional.

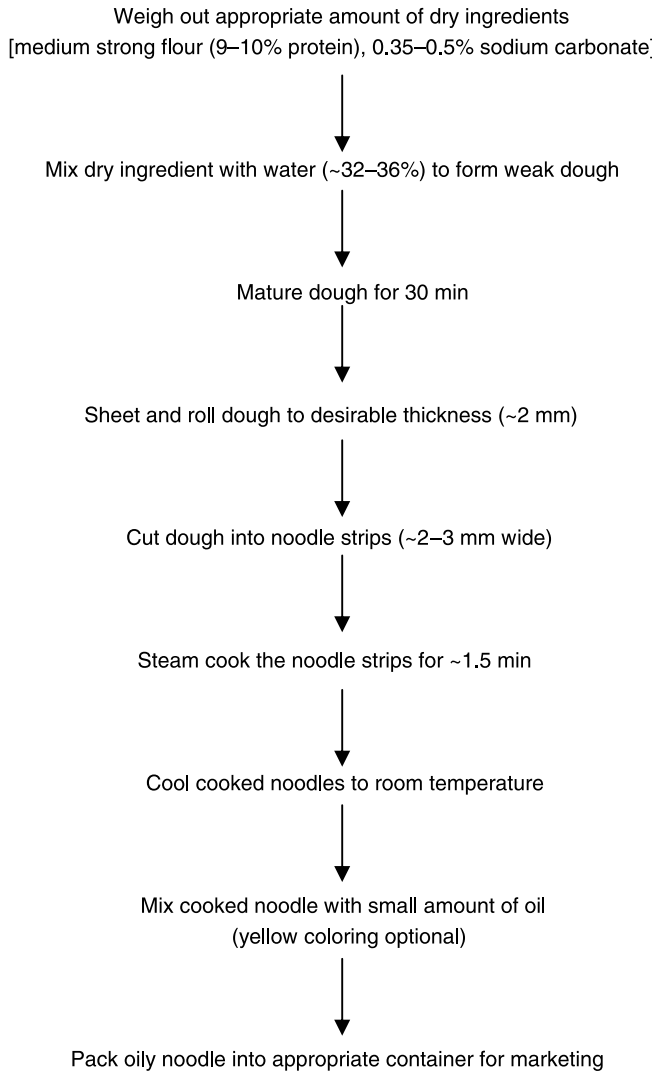

Figure 23.16 *Generalized scheme for the manufacture of oily noodles.*

Because of the absence of gluten in buckwheat, these noodles have a soft texture, but can be firm and chewy dependent on the manufacturing processes. Other ingredients such as green tea have been incorporated into some speciality buckwheat products. Dried *soba* is made by an extrusion process with uniform diameter and length. Fresh *soba* is made manually by repeated kneading and dusting of the dough to give a fairly firm chewy texture, as compared to the regular *soba* which has a fairly soft texture. Buckwheat-based Asian noodles such as *soba* are produced mainly in Japan and Korea. Typical examples are shown in Figure 23.17. They may be plain buckwheat *soba* or have flavoring added such as green tea. Buckwheat flour is also used as an optional ingredient in the manufacture of other wheat-based Asian noodles such as the cat's ear (*mou-er-dao*) noodles. Their production process is similar to regular wheat-based noodles.

Dried Japanese buckwheat soba

Korean buckwheat soba

Figure 23.17 *Examples of Buckwheat-based noodles.*

23.5 RICE-BASED ASIAN NOODLES

Rice noodles (*mi fen* or rice sticks, Fig. 23.18) differ from wheat-based Asian (oriental) noodles in that rice is the basic structural component (Table 23.1). The rice component may be wet-milled rice starch or plain rice flour. The traditional manufacture of rice noodle is a tedious procedure involving the wet milling of polished rice to remove the soluble constituents in the rice kernel, and gelatinization of the rice starch. This process also involves the problem of liquid waste disposal, although it is not high in biological oxygen demand (BOD). This procedure can be modified by using rice flour directly instead of the wet-milled rice flour, thus avoiding the liquid waste disposal problem. However, dry-milled rice flour is not the same as wet-milled rice flour (which is mainly rice starch and a small amount of rice protein), and the quality of the final product is not expected to be the same. It is generally believed that wet-milled rice flour has a

Malaysian rice sticks

Wide rice noodle

Vietnamese rice sticks

Fresh oily "ho fen"

Figure 23.18 *Examples of rice noodles.*

smoother texture after gelatinization. Figure 23.19 describes the general steps involved in the production of dry rice noodles.

Consumers should be aware that, in recent years, some manufacturers have started to replace the rice flour with other starch materials to make similar products, and still call them "rice sticks". They have similar properties, but are not as good as the original rice

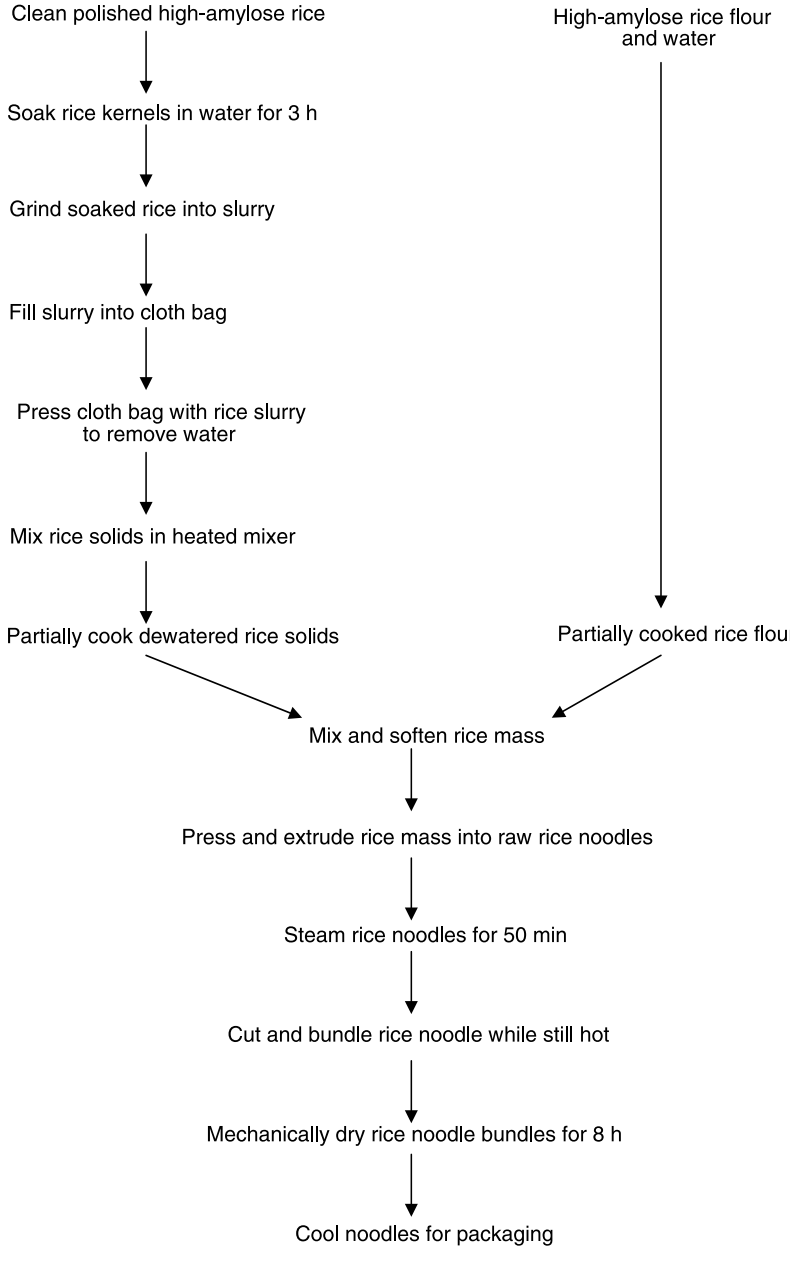

Figure 23.19 *Steps in the production of dry rice noodles.*

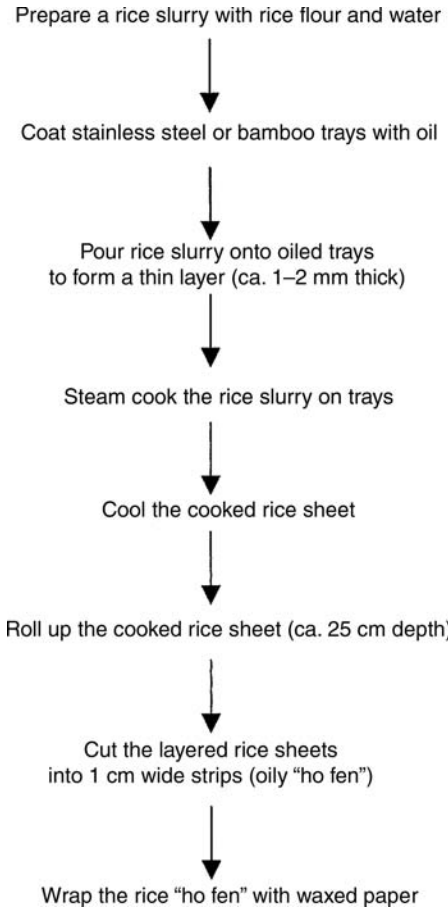

Figure 23.20 *Steps in the production of Cantonese* ho fen.

noodles (rice stick). Cost of the raw materials may be the main reason for this development. Very thin rice noodles are now made and pregelatinized by steam cooking followed by drying for the instant rice noodle market. This offers an alternative to the instant (wheat) noodle product.

The Cantonese "*ho fen* (rice stripes)" is another rice-based Asian noodle, which is an oily product. Rice-flour slurry is prepared, followed by steaming of thin layers of the slurry on oil-coated stainless trays or bamboo sheets. The gelatinized rice-flour slurry or "*fen*" is then folded into layered slabs, followed by cutting the slabs into broad strips (Fig. 23.18). Figure 23.20 describes the basic steps in the making of Cantonese "*ho fen*". They are much broader than the Chinese "hand-cut *mien*" or Japanese "*udon*". Oily rice-based "*ho fen*" is very soft and smooth in texture. The granular size of the rice flour used has a definite effect on the quality of the final product, as the difference in granular size can be detected easily. The original *ho fen* was made with wet-milled rice flour of very fine texture. However, it is much more costly to make and has a liquid waste disposal problem, as with dry rice noodles.

23.6 STARCH-BASED ASIAN NOODLES

The use of starch from various sources to manufacture products in noodle shapes has been practiced for centuries in China and subsequently spread to neighboring countries. These products are sometimes called "starch noodles" or "cellophane noodles" because of their translucent or transparent appearance before or after cooking. This transparent appearance is due to the gelatinization of starch in the manufacturing process. After drying, the product shows a translucent appearance; thus, Westerners call them "cellophane noodles". Mung bean threads or "*fen si*" or "*sai fen*", were probably the first starch noodles manufactured (Fig. 23.21). Vietnamese "*bun tau*", Korean *dang myun*, and Japanese *harusame* are similar products (Table 23.1). The best mung bean threads remain in their original shape and intact for about two days after cooking and being kept in soup. This is because of its unique starch gelling properties, which also provide very good *al dente* properties. Figure 23.22 describes the procedures used to make traditional mung bean threads. Recently, broad strips made from mainly mung bean are also available.

True (pure) mung bean threads are made from mung bean only. However, the products on the market today use other starch materials like broad beans, and other starches besides mung bean, or just plain starch only (corn starch, tapioca starch, or potato starch). Making true mung bean threads involves intensive labor and liquid waste disposal, and these are bottlenecks in the process. The liquid waste is fairly rich in nutrients as it contains all the vitamins, minerals, and proteins in the mung bean and can be used as animal feed. Attempts have also been made to recover the protein from this liquid waste. Because of these issues, it is understandable that materials other than mung bean and improvements in technology are considered. However, these products are not as good as the true mung bean threads as they become very soft, easily, upon cooking.

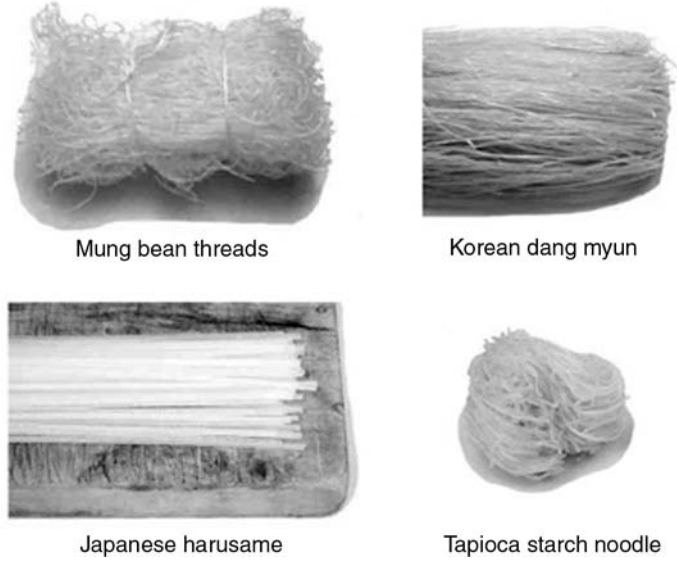

Figure 23.21 *Examples of starch-based noodles.*

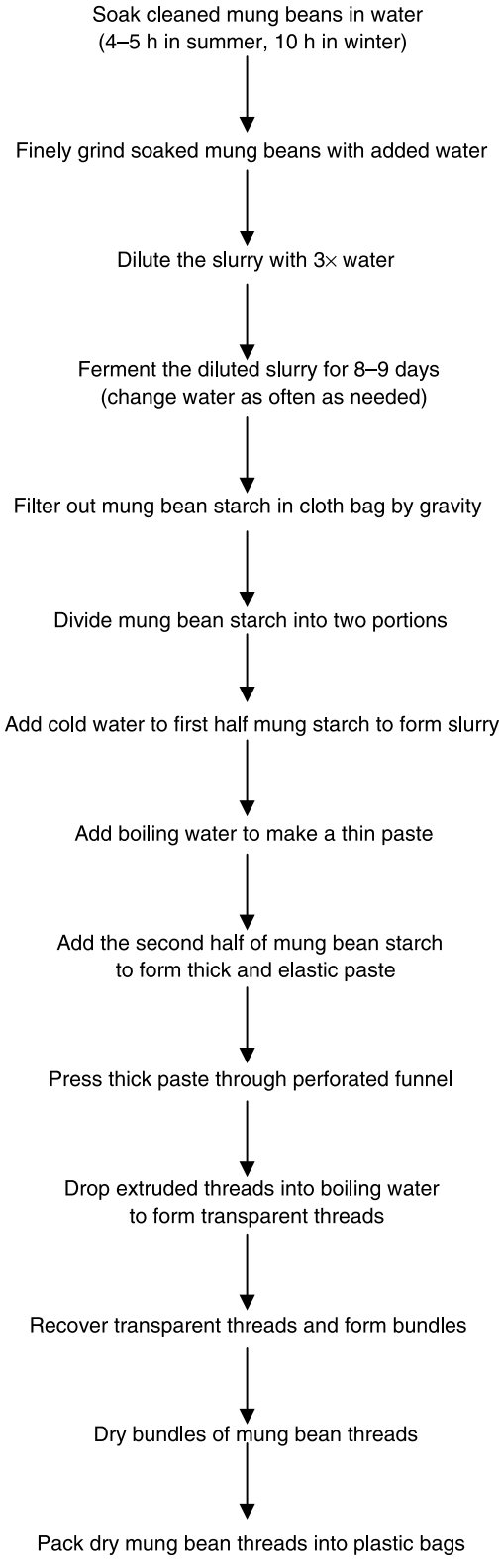

Figure 23.22 *Steps in the production of traditional mung bean threads.*

Japanese shirataki packed in water Japanese shirataki

Figure 23.23 *Japanese shirataki noodles.*

The Korean sweet potato vermicelli ("*dang myun*") is a product similar to mung bean threads, having transparency after it is cooked. Addition of other starches is also practiced. However, it is not purely colorless, but a kind of light brownish-green. It also has excellent *al dente* properties, which remain upon re-heating. They are produced as long and thick noodles. Japanese *harusame* is a similar starch noodle product, as it is also made from starches from potato, sweet potato, rice, or mung bean (Fig. 23.21).

23.7 YAM NOODLE

A very unusual Asian (oriental) noodle is the translucent Japanese "*shirataki*" noodle made from devil's tongue yam (elephant yam or konjac/konjak yam) flour. Elephant yam is used to make *Konjac/kojac* gum, a GRAS food ingredient in the United States. This *shiratake* product is marketed in a form different from others. The most common form is noodle-like, packed in liquid in a sealed container, pasteurized and kept refrigerated (Fig. 23.23). A sterilized product is also available, as are *shiratake* blocks. This product is considered a low-calorie food (only 10 calories per serving) as it utilizes mainly the gums (hydrocolloids) in the devil's tongue yam as the main structural material. Fresh elephant yam contains glucomannan (a soluble dietary fiber) and starch at a ratio of about 2 : 1. The *kojac* gum is coagulated by calcium salt(s) and the starch is gelatinized in the formation of a gel-like product with excellent water-holding capacity. It is popular in Japan and Taiwan, and also available in the oriental markets in the United States.

23.8 VARIABLES AFFECTING PRODUCT QUALITY OF ASIAN (ORIENTAL) NOODLES

In the manufacture of Asian noodles, various processing steps are applied: the selection of raw materials; mixing of ingredients to form dough, slurry, or paste; resting of dough; sheeting and rolling; extruding; shaping; pressing of slurry or paste through perforated funnels or screens; steaming, boiling, or frying; and cooling and drying. In order to be successful in the production of Asian noodles, the manufacturer has to consider, besides the processing steps, production costs, environmental issues, consumer preference, market competition, and proprietary formulations and practices.

TABLE 23.5 Variables Affecting Asian Noodle Manufacture.

Group	Variables
1. Ingredients	Amount and kinds of alkaline salts used
	Amount of coating oil used
	Amount of egg solids used
	Amount of salt used
	Buckwheat flour used
	Kinds and amount of savory ingredients used
	Kind and amount of starchy materials used
	Kinds of frying oil used
	Mung bean used
	Rice flour used (especially amylose to amylopectin ratio)
	Water quality
	Wheat flour used (especially protein content)
2. Dough quality	Rheology of dough
	Viscosity of slurry
3. Processing conditions	Cooking conditions
	Cutting actions for dough or noodle stripes
	Dough resting condition and duration
	Drying temperature, duration and condition
	Extruding condition
	Frying temperature and duration
	Mixing of ingredients
	Kneading of dough
	Relative humidity in drying chamber and environment
	Sheeting and rolling actions
	Starch extraction conditions
	Steaming temperature and duration
	Stretching actions on dough

Source: Adapted from Lu and Nip (2006).

Food technologists and scientists are more interested in the composition of ingredients, the kinds of oil used and amount absorbed, the application of food additives to prolong shelf-life, the rheology of dough, consistency of slurry, work needed to knead the dough, and others (Anon. 1995; Azudin 1998; Baik and others 1994, 1995; Bejosano and Corke 1998; Bhattcharya and Corke 1996; Crobie 1990; Dexter and others 1979; Edwards and others 1996; Galvez and others 1994; Hatcher and others 1999; Jin 1956; Kim and Seib 1993; Konik and others 1993, 1994; Lu and Kuo 1991; Lu and others 1991; Lu 1990; Miskelly and Moss 1985; Moss and others 1986, 1987; Oda and others 1980; Oh and others 1983, 1985, 1986; Ross and Hatcher 2005; Rho and others 1986; Shelke and others 1990; Toyokawa and others 1989a,b; Vadlamani and Seib 1996). The major variables that could affect the quality of Asian (oriental) noodles based on the literature published are summarized in Table 23.5. These published data do not answer all the questions a food technologist or scientist wants to ask, but will provide some indications on what is known and what needs to be studied in the future.

23.9 CONCLUSIONS

Beginning with primitive cottage-type industry, Asian noodle manufacture is now a big industry, especially for instant noodles. With the migration of various ethnic peoples to

various parts of the world and cultural exchange, it has captured the attention of consumers worldwide. It is expected that this industry will grow even further with improvement in manufacturing technology, and will be accepted further by more consumers in various regions.

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Section VII

Cheeses

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

24

Cheddar and Related Hard Cheeses

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24.1 Introduction 568
24.2 Definitions 569
24.3 Production of Cheddar and Related Hard Cheeses 570
24.3.1 Ingredients 570
24.3.1.1 Milk 570
24.3.1.2 Calcium Chloride 571
24.3.1.3 Starter Cultures 572
24.3.1.4 Adjunct Cultures 572
24.3.1.5 Color 573
24.3.1.6 Enzymes 573
24.3.1.7 Salt 573
24.3.1.8 Other Optional Ingredients 573
24.3.2 Preparations for Cheesemaking 574
24.3.2.1 Culture Selection and/or Propagation 574
24.3.2.2 Cheese Milk Pretreatments 574
24.3.2.3 Homogenization 575
24.3.2.4 Pasteurization 575
24.3.3 Cheesemaking 576
24.3.3.1 Calcium Chloride Addition 576
24.3.3.2 Color Addition 577
24.3.3.3 Culture Addition 578
24.3.3.4 Ripening 578
24.3.3.5 Enzyme Addition 578

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

24.3.3.6 Cutting 579
24.3.3.7 Healing 579
24.3.3.8 Cooking 580
24.3.3.9 Draining 582
24.3.3.10 Washing 582
24.3.3.11 Cheddaring 582
24.3.3.12 Milling 583
24.3.3.13 Salting 583
24.3.3.14 Pressing and Packaging 583
24.3.3.15 Aging 584
24.4 Quality Control 584
24.4.1 Shelf-life 584
24.4.2 Evaluation 584
24.4.3 Safety 586
24.5 Trouble-Shooting 587
24.5.1 No Curd or Weak Curd Formation 587
24.5.2 Natural Inhibitors 587
24.5.3 Antibiotics 587
24.5.4 Residual Cleansers/Sanitizers 588
24.5.5 Bacteriophage 588
24.5.6 Milk Pretreatment 588
24.6 Crystal Formation 589
References 590

Cheddar, oh Cheddar, what could be better?
Crackers are naked not topped with a slice.
Laid on fresh bread makes a sandwich that's nice.
Waxed or canned, sharp is the best.
Omelets are bland not filled with some shreds.
Cheddar, dear Cheddar, what could be better?
Without you to eat, my meals aren't complete.
—Anonymous

24.1 INTRODUCTION

No book about dairy products is complete without a chapter dedicated to Cheddar cheese. Cheddar cheese originated in the village of Cheddar, in Somerset, England, in the nineteenth century (Banks and Williams 1997a, 2004). The term, "Cheddaring" specifies the process of piling and re-piling of blocks of warm curd into cheese vats. During the Cheddaring period of about 2 h, lactic acid increases rapidly and the proteins stretch and align, which results in the body and texture characteristic of Cheddar cheese. The first Cheddar cheese factory in the United States, other than farmhouse cheesemaking, was established in New York in 1861 (Lawrence and Gilles 1987a). The procedures for Cheddar cheese manufacture were popularized in America in 1876 by Robert McCadam, leading to the evolution of the American Cheddar cheese industry (Kosikowski and Mistry 1997a).

The United States is the largest producer of Cheddar cheese in the world. In 2001, production of Cheddar cheese in the United States exceeded 1.2 billion kg and supermarket

sales surpassed 219 million kg (IDFA 2002). Today, consumers eat approximately 4.6 kg of Cheddar cheese per capita (IDFA 2005). The greatest centers of American cheese production (including Cheddar) in 2001 were Wisconsin (1 billion kg), followed by California (723 million kg), New York (318 million kg), Minnesota (269 million kg), and Idaho (257 million kg) (IDFA 2002). Today, Idaho leads New York and Minnesota (IDFA 2005) in overall natural cheese production because of its high Italian cheese production.

Extensive modifications in Cheddar cheese manufacture have taken place with the introduction of continuous Cheddar cheese manufacturing systems in large establishments. However, regardless of advances in automation, starter cultures are always used in the manufacturing of Cheddar cheese and considerable attention is given to culture selection. The primary function of starter cultures is to produce acid during the fermentation process, but starters also contribute to cheese ripening, as their enzymes contribute to proteolysis and the formation of flavor compounds (Wallace and Fox 1997).

This chapter will describe similarities and differences among Cheddar and related hard cheeses, outline manufacturing steps involved in production, and describe technological advances in the production of cheeses in the Cheddar and Cheddar-type cheese category.

Please pass the cheese, Louise
Louise, please pass the cheese
The ham and spread
Are on the bread
I'm lacking Cheddar cheese
—Anonymous

24.2 DEFINITIONS

Cheddar cheese (FDA 2004c) is classified as a hard cheese, ranging in color from nearly white (particularly if made from goat or sheep milk) to yellow to orange (USDA 1978). Standards of identity for Cheddar include $\leq$39% moisture and $\geq$50% fat on a dry basis (FDA 2004e). Low-sodium Cheddar cheese contains not more than 96 mg of sodium per 454 g finished food.

Cheeses closely related to Cheddar include Longhorn, Colby (FDA 2004d), and Monterey Jack (FDA 2004h). Although a Cheddar is traditionally about 36.83 cm in diameter, 30.48 cm thick, and weighs between 31.75 and 35.38 kg, Longhorn is 15.24 cm in diameter (round), 33.02 cm long, and weighs 5.5 to 6 kg (USDA 1978). Longhorn is not separately defined in the Code of Federal Regulations, but it may be found commercially. Longhorn Cheddar is essentially a name that describes the round shape derived from Longhorn hoop usage during the pressing step.

Colby manufacture resembles Cheddar except that the curd is "washed" and stirred instead of matted and milled (Kosikowski and Mistry 1997a). Colby is moister, softer, and more open in texture than Cheddar. Moisture must not exceed 40% and fat in the solids must be at least 50% (FDA 2004d). Colby contains between 1.4 and 1.8% salt (USDA 1978).

Monterey, Jack or Monterey Jack cheese was first made in Monterey County, California, in 1892 (USDA 1978). Monterey is made in a similar fashion to Colby, but the procedure requires less time. Monterey contains more moisture and is softer than Cheddar and Colby (USDA 1978). Standards of identity state that Monterey must

contain not more than 44% moisture and at least 50% fat in the solids (FDA 2004h). High-moisture Jack cheese conforms to the definition and standard of identity and is subject to the requirement for label statement of ingredients prescribed for Monterey cheese, except that its moisture content is more than 44% but less than 50% (FDA 2004h). Monterey typically contains 1.5% salt (USDA 1978).

24.3 PRODUCTION OF CHEDDAR AND RELATED HARD CHEESES

Good cheese requires high-quality milk and carefully selected starter cultures. However, additional ingredients are often utilized to enhance visual appeal (annatto), coagulation properties (calcium chloride or enzymes), and flavor development (adjunct cultures or enzymes) to make great cheeses. How well the additional steps are employed determines whether or not one makes a great cheese. The following section will elaborate on individual ingredients and their function in cheesemaking.

24.3.1 Ingredients

24.3.1.1 Milk. To make high-quality cheese, producers must start with high-quality milk. Cheese quality will never be better than the starting materials. Cheddar and most Cheddar-like cheeses can be made from raw, heat-treated, heat-shocked, or pasteurized milk, nonfat milk or cream, alone or in combination (FDA 2004c). Legally, there is no distinction between raw and heat-treated/shocked milk. Because of the potential for pathogens to survive in cheese for up to 60 days, cheeses made from raw or heat-treated/shocked milk must be aged for at least 60 days at greater than 1.66°C prior to sale (FDA 2004e). Cheeses must be aged at greater than 1.66°C to ensure microbial metabolic activity and progression through the life and death cycle. Cheeses made from pasteurized milk need not be aged prior to sale. Monterey Jack cheese milk must be pasteurized because of its limited aging (FDA 2004h).

Regardless of use of heat treatment, low bacteria counts are essential for high-quality cheese, as high bacterial counts can lead to flavor and body defects. Psychrotrophic bacteria, including *Pseudomonas*, *Aeromonas*, *Flavobacterium*, *Acinetobacter*, *Bacillus*, *Micrococcus*, and other genera, can grow relatively rapidly in milk maintained at 7°C or lower (Frank and Marth 1988; Richard and Desmazeaud 2000; Banks and Williams 2004), so extended storage of milk (beyond 48 h) prior to pasteurization or cheesemaking is highly discouraged. Enzymes produced by psychrotrophs, including heat-stable lipases and proteinases, can act directly on milk proteins and lipids, reducing yield and contributing to quality defect development in the resultant cheese (Johnson 1988; Richard and Desmazeaud 2000).

Extended storage of milk prior to pasteurization and cheesemaking not only enables growth of psychrotrophic bacteria, but also encourages solubilization of colloidal calcium phosphate and a shift in caseins from the micellar to soluble state (Johnson 1988). Although soluble caseins constitute less than 15% of the total casein in normal milk directly from the udder, the proportion increases to up to 42% of total casein during storage at 4°C (Johnson 1988). Soluble calcium phosphate and casein are lost during whey drainage, which reduces cheese yield (Johnson 1988).

TABLE 24.1 Recovery of Milk Components in Cheddar Cheese.

Constituent	Percent in Cheese-Milk	Percent Recovered in Cheese
Water	87.0–88.0	4.5
Fat	3.0–4.5	92.5
Casein	3.0–4.0	96.0
Lactose	4.5–5.0	4.0
Whey protein/salts	1.2–1.8	29.0

Because approximately 90% of both fat and protein from cheese milk are captured in the cheese (Table 24.1), and these components make up 91% of the solids in cheese (Johnson 1988), detrimental effects on either component will be realized in the cheese yield and quality. Variability in milk composition is discussed in another chapter of this book. For consistency in yield and product composition, milk for Cheddar cheese is commonly standardized to a casein-to-fat ratio between 0.67 and 0.72 (Lawrence and Gilles 1987a; Banks and Williams 2004).

Somatic cells also have an impact on cheese yield. Barbano and others (1991) demonstrated that milk casein as a percentage of true protein (C%TP) and cheese yield efficiency were lower when milk somatic cell count (SCC) was high. Cheese moisture, as well as fat and protein losses in whey, increased with increased SCC (Barbano and others 1991). It was concluded that any increase in milk SCC above 100,000 cells/mL negatively affects cheese yield for milk from groups of cows with similar milk SCC (Barbano and others 1991).

Raw milk naturally contains low levels of endogenous enzymes, including alkaline phosphatase, plasmin, and lipoprotein lipase (Whitney 1988). Alkaline phosphatase (ALP) is slightly more heat stable than the most heat-resistant pathogenic microorganisms in milk. Thus, ALP is a convenient indicator of pasteurization. Indeed, the Pasteurized Milk Ordinance defines legal limits for ALP for Grade "A" pasteurized milk and bulk shipped heat-treated milk products (U.S. Department of Health and Human Services 1999 revision). ALP assays should also measure negative in cheeses made from pasteurized milk. Plasmin, which is stable at pasteurization temperature, hydrolyzes both β- and α_{s1}-casein in milk and in cheese during maturation, thus contributing to cheese maturation (Johnson 1988; Banks and Williams 2004).

The lipoprotein lipase found in milk is identical to the lipoprotein lipase in blood and represents a spillover from the mammary tissues (Weihrauch 1988). Lipoprotein lipase, if activated by severe agitation, temperature fluctuations, or other means, leads to hydrolysis of fatty acids from triacylglycerides and rancid off-flavors in milk and the subsequent cheese. Rancidity in milk may be detectable at acid degree values exceeding 1.2 meq/L (Bodyfelt and others 1988; Weihrauch 1988). Raw milk is somewhat resistant to lipase because of the protective nature of the milkfat globule membrane (Weihrauch 1988). Unlike contaminating bacterial lipases, natural milk lipase is heat labile. Heating of milk to 80°C for 20 s destroys all lipases in milk (Weihrauch 1988).

24.3.1.2 Calcium Chloride. Calcium chloride may be added to the cheese milk as a coagulation aid, in an amount not more than 0.02% (calculated as anhydrous calcium chloride) of the weight of the dairy ingredients. Addition of calcium chloride reduces

the coagulation time and increases curd firmness (Lenoir and others 2000b). Calcium added to milk is solubilized during acidification and thus lost in whey, so it does not contribute to total calcium in the final cheese.

24.3.1.3 Starter Cultures. Starter bacteria can be defined as isolates that produce sufficient acid to reduce the pH of milk to <5.3 in 6 h at $30-37^{\circ}C$, and aid in curd digestion and flavor development (Lewis 1987). Cultures selected for Cheddar cheese made throughout the world are typically "O" cultures, which are designated as cultures that produce lactic acid from lactose. The "O" type cultures are composed of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Harrits 1997; Strauss 1997). *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* can be differentiated by their ability to grow at $40^{\circ}C$ and in the presence of salt. Whereas *L. lactis* subsp. *lactis* will grow at $40^{\circ}C$ and in the presence of 4% salt, *L. lactis* subsp. *cremoris* will not grow at $40^{\circ}C$ and will grow in the presence of salt up to a 2% concentration (Harrits 1997). The "L" type cultures consist of "O" type cultures plus the citrate-fermenting culture *Leuconostoc mesenteroides* subsp. *cremoris*, which produces flavor compounds (e.g., diacetyl) plus small amounts of carbon dioxide (Harrits 1997). "L" type cultures may also be used in the production of Colby, because an open structure is allowed in Colby (Harrits 1997). Ideally, acid should be formed quickly and at a steady rate during curd formation. Bacteriophage (phage) resistance, salt sensitivity, and protease activity (desirable flavor development) are additional selection criteria (Strauss 1997). Homofermentative starter cultures are added deliberately to initiate Cheddar cheese manufacture. The starter bacteria produce L(+)-lactate from lactose and they grow, typically attaining cell densities of 10^8 cfu/g within hours of the beginning of manufacture. L(+) refers to an optically active substance that rotates the plane of polarized light counterclockwise (also called levorotatory). The optical isomer of L(+)-lactate is D(−)-lactate. The optical isomers are mirror images of each other and result from the tetrahedral geometry around the chiral carbon center.

Production of homogeneous, high-quality Cheddar cheese requires uniform lactose fermentation, lipolysis, and proteolysis, each of which varies among bacterial strains. A relationship the exists between the extent of starter cell autolysis and the level of lipolysis during Cheddar cheese ripening (Collins and others 2003). The rate and extent of both fermentation and proteolysis depend upon temperature and salt concentration (Thomas and Pearce 1981). One of the main roles of starter bacteria is to provide a suitable environment for enzyme activity from rennet/chymosin (an acid protease) and favorable growth of secondary microflora with respect to redox potential, pH, and moisture content in cheese. Redox potential is a measure of the tendency of a system to donate or accept electrons, and indicates aerobic or anaerobic conditions. Typically, the environment inside cheese is anaerobic and reducing. Depending upon the type of culture preparation, usage rates vary between 0.75 and 1.25% for traditional bulk starter and 0.5% and 0.6% for pH controlled starter or DVS starter, respectively (Strauss 1997).

24.3.1.4 Adjunct Cultures. An adjunct culture is a one that is added, along with starter culture, for the desirable characteristics it may impart upon the cheese other than acid. Adjunct cultures are select nonstarter lactic acid bacteria (NSLAB), because not all NSLAB are desirable. Although the specific ripening mechanisms of NSLAB that contribute positively to Cheddar flavor have not been fully determined, to be successful, NSLAB adjuncts require two important features (Crow and others 2001). First, strains

must provide a balance of beneficial ripening reactions in cheese (Crow and others 2001). Secondly, strains need to be competitive against adventitious (not intentionally added) NSLAB and remain the dominant NSLAB during the ripening period (Crow and others 2001).

Lactobacillus helveticus is one species that may be added to Cheddar cheese milk for desirable cheese flavor development. *L. helveticus* species tend to be thermophilic, proteolytic, and have the ability to utilize galactose after other sugars are fermented (Harrits 1997). Because of their proteolytic capabilities, *L. helveticus* adjunct cultures have shown themselves to effectively improve the sensory quality of reduced-fat Cheddar cheese (Drake and others 1997).

24.3.1.5 Color. The recognizable yellow to orange color of Cheddar and Colby cheeses is derived from annatto, an extract from seeds of the "Lipstick tree," *Bixa orellana* (Walstra and others 1999). Annatto was first used to make cheeses appear more fat-rich, when made during seasons of the year when the milk of cows produced less colorful cheese because the cows were fed diets lower in beta-carotene. Sheep and goat milk cheeses are naturally whiter than cow milk cheeses because the beta-carotene is efficiently hydrolyzed to vitamin A in the digestive tracts of these species.

24.3.1.6 Enzymes. The Code of Federal Regulations permits the use of rennet/chymosin and/or other clotting enzymes of animal, plant, or microbial origin, as well as enzymes of animal, plant, or microbial origin, used in curing or flavor development for Cheddar cheese (FDA 2004j). Enzymes of starter, adjunct NSLAB, and adventitious NSLAB naturally contribute to flavor and body/texture development in cheeses. Kheadr and others (2003) were able to accelerate Cheddar cheese proteolysis and lipolysis using various liposome-encapsulated enzymatic cocktails. A neutral bacterial protease, acid fungal protease, and lipase were individually entrapped or mixed as cocktails and entrapped in liposomes then added to cheese milk prior to renneting (Kheadr and others 2003). Certain enzyme treatments resulted in cheeses with more mature texture and higher flavor intensity or Cheddar flavor in a shorter time compared with control cheeses (Kheadr and others 2003).

24.3.1.7 Salt. Cheddar cheese typically contains 1.6–1.8% salt (Williams and others 2000), in the form of NaCl. Salt enhances flavor, encourages syneresis, and slows or stops growth of salt-sensitive bacteria. Food-grade salt is essential to the production of safe, high-quality Cheddar cheese, and consistent salt grain size contributes to uniformity of salt concentration throughout the cheese matrix.

Investigators (Reddy and Marth 1993, 1995a,b) have demonstrated that reduced-salt, or low-salt Cheddar cheese can be made by replacing sodium chloride (NaCl) with potassium chloride (KCl) or mixtures of the two salts. Use of KCl to replace some of the NaCl for salting cheese has no detectable effect on the kinds of lactic acid bacteria, aerobic microorganisms, aerobic spores, coliforms, and yeasts and molds in cheeses when compared with control cheeses (Reddy and Marth 1993). Authors concluded that low-sodium Cheddar cheese can readily be produced without affecting its composition when one-third or more of the NaCl added to cheese curds is replaced with KCl (Reddy and Marth 1993).

24.3.1.8 Other Optional Ingredients. The Code of Federal Regulations allows the use of antimycotic agents, applied to the surface of slices or cuts in consumer-sized packages (FDA 2004a). Some of the antimycotic substances allowed by the FDA are calcium propionate (FDA 2004b), methylparaben (methyl *p*-hydroxybenzoate) (FDA

2004g), propylparaben (propyl *p*-hydroxybenzoate) (FDA 2004i), sodium benzoate (FDA 2004k), sodium propionate (FDA 2004l), and sorbic acid (FDA 2004m).

Hydrogen peroxide is allowed, if followed by a quantity of catalase preparation sufficient to eliminate the hydrogen peroxide. The weight of the hydrogen peroxide shall not exceed 0.05% of the weight of the milk (FDA 2004f) and the weight of the catalase shall not exceed 20 ppm of the weight of the treated milk (FDA 2004c).

24.3.2 Preparations for Cheesemaking

24.3.2.1 Culture Selection and/or Propagation. Culture quality is of the utmost importance in the production of high-quality cheese. Culture manufacturers commonly work closely with processing facility operators to effectively meet specific needs. Culture manufacturers continuously develop unique culture combinations for a given product. Several types of culture forms are available, including

1. Liquid (for propagation of mother culture; rarely used today);
2. Deep-frozen concentrated cultures (for propagation of bulk starter);
3. Freeze-dried concentrated cultures in powder form (for propagation of bulk starter or DRI-VAC, for preparation of mother culture); and
4. Deep-frozen or freeze-dried, superconcentrated cultures in readily soluble form for direct inoculation of the product (direct vat set, DVS).

The availability of frozen or freeze-dried cultures eliminates the need for small dairy plants to make cultures or operate a culture room (Lewis 1987). The culture room is a separate room in the dairy plant reserved for preparation and propagation of starters and an important element in production of quality cheese because it limits opportunities for contamination by airborne yeast, mold, and bacteriophage (Lewis 1987). Bacteriophage (phage) are viruses that infect specific strains of bacteria, which stresses the importance of utilizing mixed-multiple strains and starter culture rotation in cheesemaking. Proliferation of phage will result in a failure of lactic acid production, termed "stuck vat," necessitating strict sanitation and whey-handling practices to keep phage numbers to a minimum. Larger plants are typically supplied with frozen or freeze-dried cultures for the manufacture of bulk starters in aseptic bulk culture rooms (Lewis 1987). Cooling and storage conditions and shelf-lives of cultures vary. Generally, deep-frozen and freeze-dried cultures can be stored for 9–12 months at $-18^{\circ}C$ and $-45^{\circ}C$, respectively (Lewis 1987). Maintenance of consistency across cheese lots requires constancy of culture handling.

24.3.2.2 Cheese Milk Pretreatments. Centrifugal clarifier-separators are used to separate the cream and skim fractions, as well as remove solid impurities from milk prior to standardization. Cheese milk fat and protein content are commonly standardized for consistency of yield and composition. Fat may be increased with the addition of cream, and protein, particularly casein, may be increased with nonfat dry milk, skim milk, or condensed skim milk (Johnson 1988). A typical casein-to-fat ratio of between 0.67 and 0.72 may be used (Lawrence and Gilles 1987b; Banks and Williams 2004). Although cheese moisture is influenced by numerous factors during cheesemaking, higher fat levels in cheese milk are typically associated with lower moisture cheeses (Lawrence and Gilles 1987b). As a general rule, an increase of 0.05 in the casein-to-fat ratio in milk generally

results in a decrease of about 1.4% in the fat on a dry basis and an increase of about 0.8% in moisture in Cheddar cheese (Lawrence and Gilles 1980).

Most commonly in the United States, whole milk is preheated to 55–65°C in the regeneration section of the high-temperature short time (HTST) pasteurizer prior to separation. Following separation, the cream is standardized to a preset fat level and the fraction intended for standardization of milk is routed and remixed with the proper amount of skim milk to attain the desired fat and protein content. The surplus cream is directed to a separate cream pasteurizer, and the standardized milk flows through the pasteurizer.

El-Gazzar and Marth (1991) recommended the use of ultrafiltered milk for conversion into such cheeses as Cheddar, cottage, havarti, feta, brick, Colby, and Domiati because of an increase in yield of product. Ultrafiltration results in the concentration of milk proteins, with reduction in lactose and mono- and divalent cations. Additional benefits claimed for use of ultrafiltered milk in cheesemaking include reduction in costs of energy, equipment, and labor, improved consistency of cheese flavor, and the potential production of new byproducts (El-Gazzar and Marth 1991).

24.3.2.3 Homogenization. Although homogenization of cheese milk is typically not employed in the production of Cheddar cheese, research has shown that homogenization of cream may have applications to Cheddar cheese. Homogenization is most efficient when fat globules are in the liquid state, so milk is preheated in the plate heat exchanger in the regeneration section of the HTST pasteurizer, where the temperature is raised to at least 60°C prior to homogenization (Morr and Richter 1988). In a two-stage homogenizer, pressures typically range from 10 to 25 MPa in the first stage and 5 MPa in the second stage.

In a study with Cheddar cheese standardized to a casein-to-fat ratio of 0.70, Nair and others (2000) demonstrated that cheese hardness was not influenced by homogenization, and cheeses with homogenized cream had improved body and texture and flavor over controls. Cream homogenized at 6.9 MPa (first stage) and 3.5 MPa (second stage) was optimal for enhancing Cheddar cheese yield and functionality (Nair and others 2000). Metzger and Mistry (1994) demonstrated that cheese moisture and yield were higher in reduced-fat Cheddar cheeses made with homogenized cream than controls. The body and texture of reduced-fat Cheddar cheeses made from homogenized cream were improved over those for the control cheeses, which were hard, rubbery, and curdy (Metzger and Mistry 1994).

24.3.2.4 Pasteurization. The only step in the dairy processing system that guarantees the killing of pathogenic microorganisms is pasteurization. Thus, pasteurization may be considered the most critical segment of the cheese processing line. An added side-benefit of pasteurization is that it also kills many spoilage microorganisms and inactivates enzymes that may contribute to quality defects in cheese. Pasteurization contributes to consistency in product quality. Of course, strict sanitation is critical up to and beyond pasteurization to assure the safety and quality of dairy products. In HTST pasteurization, milk must be held at a temperature of at least 72°C for a minimum of 15 s to be legally pasteurized (U.S. Department of Health and Human Services 1999 Revision). In batch or low-temperature long time (LTLT) systems (uncommon in large-scale operations), milk is continuously agitated in a single tank, at a set temperature (legally at least 62.8°C) for a given time (legally at least 30 min if at 62.8°C) to guarantee inactivation of pathogens (U.S. Department of Health and Human Services 1999 Revision). Any lower temperature or shorter time than legal pasteurization means the cheese must be treated as if made from raw milk, which means products must be aged for at least 60 days at 1.66°C or higher.

Regardless of pasteurization method, cheese milk is then cooled, either to incubation temperature for selected starter cultures, or to refrigeration temperature for future applications.

Although all pathogens and most spoilage microorganisms are killed by pasteurization, potentially beneficial or flavor-producing microorganisms are also killed. And so, although cheeses made from pasteurized milk are safe, they also have less flavor than raw milk cheeses. Buchin and others (1998) studied the effects of pasteurization and fat makeup of experimental semihard cheeses with two different fat compositions. The raw-milk cheeses had more intense flavor and volatile compounds than the pasteurized milk cheeses. Raw-milk cheeses were characterized by higher amounts of numerous alcohols, fatty acids, and sulfur compounds, and pasteurized-milk cheeses were characterized by higher amounts of ketones (Buchin and others 1998). The differences were attributed to the high level of indigenous microflora in raw milk cheeses (Buchin and others 1998).

In addition to modifying milk microflora, pasteurization acts upon milk protein chemistry to influence cheese quality. Specifically, pasteurizing cheese milk influences the extent and characteristics of proteolysis during Cheddar cheese aging (Lau and others 1991). Pasteurization causes heat-induced precipitation of whey proteins upon casein micelles that results in retention of additional whey protein in the cheese beyond that which is soluble in the aqueous phase of raw-milk cheese. The presence of heat-denatured whey protein in cheese may influence the accessibility of caseins to proteases during cheese aging, a consequence of which would be differences in proteolysis during aging. These differences may be another factor that contributes to differences in flavor development in Cheddar cheese made from pasteurized and raw milk (Lau and others 1991). Temperatures higher than legal pasteurization (80°C) may be used to increase yield; however, gelling takes longer, the firming rate of the gel as well as its maximum firmness are reduced, and gel draining is more difficult and is incomplete (Lenoir and others 2000b). These factors are a consequence of denatured whey proteins, particularly β-lactoglobulin, which bind with caseins, particularly κ-casein. Indeed, a complex between β-lactoglobulin and κ-casein leads to modification in the conformation of the κ-casein chain at the chymosin cleavage site, detrimentally affecting coagulation properties (Lenoir and others 2000b). Denaturation of whey proteins is negligible at pasteurization temperatures, but reaches 10% after a treatment of 75°C for 15 s and 20% after 85°C for 30 s (Lenoir and others 2000b). Heat also decreases soluble calcium, ionized calcium, and soluble inorganic phosphorus (Lenoir and others 2000b).

24.3.3 Cheesemaking

A schematic diagram outlining the steps of cheesemaking, from fresh milk through aging cheese is shown in Figure 24.1. In large automated plants, cheesemaking is typically held to a well-timed schedule. Culture, $CaCl_2$, and color are typically added as cheese milk enters the cheese vat, after pasteurization and cooling. Chymosin is commonly added after the vat is completely filled with pasteurized milk. In pilot or small-scale operations, culture, $CaCl_2$, and color are commonly added when an entire vat of cheese milk reaches target temperature. Chymosin is added after a ripening period of 15–30 min.

24.3.3.1 Calcium Chloride Addition. If $CaCl_2$ is to be added to the cheese milk, typically 0.2% of cheese milk weight is adequate to improve coagulation properties (Kosikowski and Mistry 1997a; Lenoir and others 2000a).

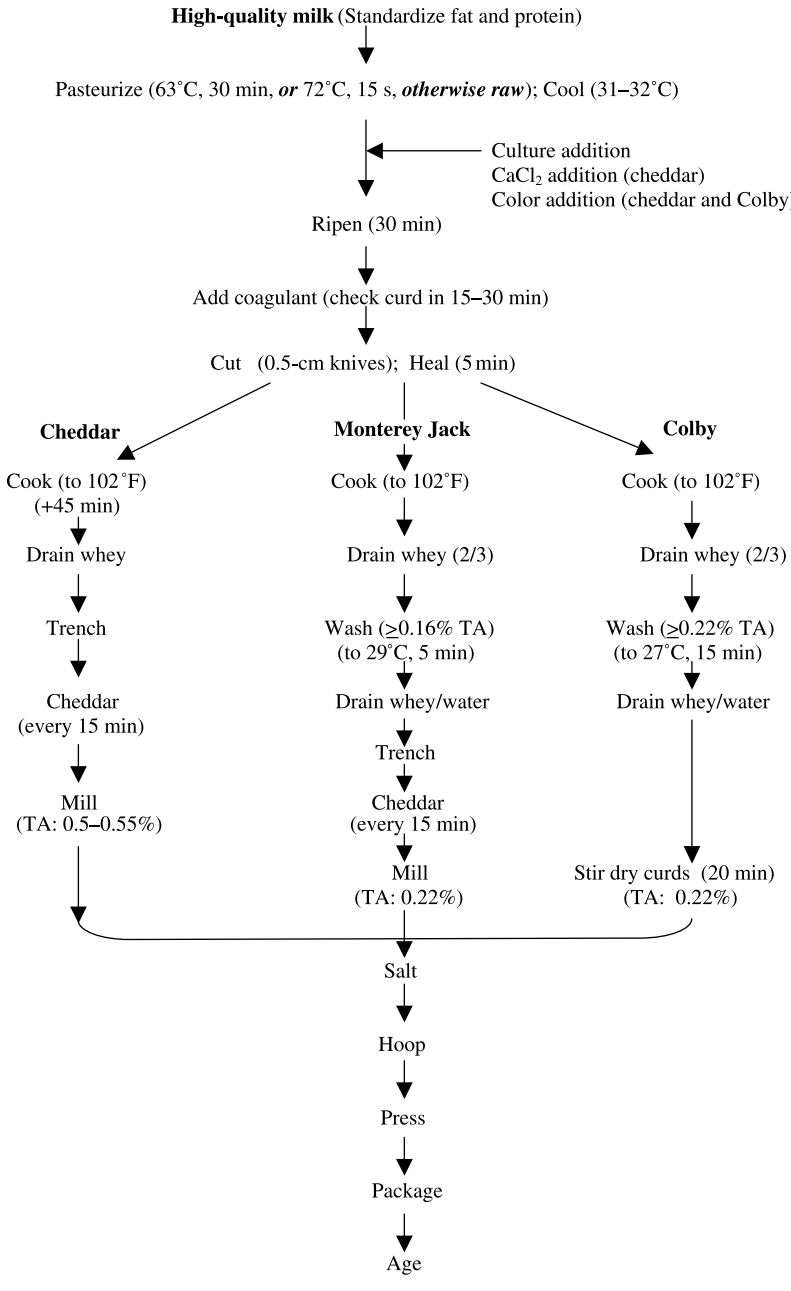


Figure 24.1 *Schematic of the steps in cheesemaking.*

24.3.3.2 Color Addition. Annatto may be added to cheese milk at a rate of approximately 66 mL per 1000 kg of milk, adjusted to desired product color (Kosikowski and Mistry 1997b). Annatto binds with protein to form a straw to orange color in the final cheese, upon a concentration that occurs with whey expulsion (syneresis).

24.3.3.3 Culture Addition. Prior to culture addition, raw or pasteurized milk must be tempered to the appropriate temperature for starter culture multiplication, approximately 26–30°C for Cheddar and related cheeses (Banks and Williams 2004). Inoculum level is defined by culture manufacturers, based upon whether the culture is DVS or bulk culture, typically from 0.5 to 5%. Cheese manufacturers may increase or decrease the amount of culture based on seasonal variation in milk composition (Lawrence and others 1999). Optional addition of adjunct culture typically varies from 0.1 to 1%.

24.3.3.4 Ripening. The titratable acidity (TA) of fresh milk is approximately 0.14–0.18, depending on composition, and pH is about 6.6–6.8. When starter culture is added, cultures need time to equilibrate to their environment (lag phase), so only a small rise in TA is noted during the 30-min ripening period. Little lactose is converted to lactic acid during the lag phase of the cultures, but TA rises steadily during the log phase of growth, during which time culture numbers increase exponentially. Even after lactic acid formation begins, little change in pH is noted because of milk's high buffering capacity, owing to the presence of proteins, citrate, and phosphate in milk.

24.3.3.5 Enzyme Addition. In fresh fluid milk, charges on the κ-casein "hairs" are negative (−), so casein micelles repel each other. With the production of acid, the charges on some κ-casein hairs begin to change to positive (+). When pH declines to near 5.2, calcium and phosphorus are solubilized and the micelle structure changes (Brulé and others 2000). At pH close to 4.6, coagulation occurs, as repulsive charges are neutralized and micelles come into contact with one another and coalesce (Brulé and others 2000). Some cheeses are made exclusively with acid coagulation (e.g., cottage cheese). Because acid development is slow, cheese make procedures that rely entirely on acid coagulation are in the order of 10–18 h in length. The cheesemaking process is accelerated by the use of coagulating enzymes.

Chymosin, originally derived from the abomasum of milk-fed calves, but now microbially or fungally derived, is the most common coagulating enzyme used in the manufacture of Cheddar and related varieties (Ramet 2000b). Chymosin is an acid protease, which means that it is more active at an acid pH than neutral or basic pH. Highest activity is observed at pH 5.5 and 42°C (Ramet 2000b). Specifically, chymosin cleaves the peptide bond Phe105–Met106, which leads to the formation of κ-para-casein (l-105) and glycomacropeptide or caseinomacropeptide (CMP, 106–169) (Brulé and others 2000). CMP is soluble in whey. When chymosin is added to milk, coagulation occurs in three steps:

1. κ-Casein hydrolysis,
2. Aggregation of destabilized micelles, and
3. Reorganization of calcium phosphate, or reticulation (Brulé and others 2000).

The coagulation process is shortened because rennet/chymosin cleaves the negatively-charged κ-casein hairs off the micelles, enabling approach and coagulation of micelles. During the coagulation process, calcium phosphate bridges form between micelles, and tighten as whey is expelled, forming a tight network of casein, which entraps some fat, water, and water-soluble components. As fermentation proceeds, Ca^{2+} are replaced by H^{+} and the casein network continues to tighten.

Approximately 5–50 mL of single-strength liquid chymosin should be used to coagulate 100 L milk (Brulé and others 2000). Chymosin should always be diluted (approximately 1 part to 40 parts water) prior to addition to the cheese vat to prevent localized coagulation. Dilution should always be done with cool (or room temperature) water immediately before adding to milk. Chymosin begins to lose its strength and activity immediately upon dilution, which is why dilution should not be done in advance. Also, chymosin is degraded by high temperatures and chlorine. Chymosin must not be overmixed into the milk because cleavage of κ-casein from casein micelle proteins begins immediately. In small operations, chymosin should only be mixed into milk for about 1 min to maximize yield. During the incubation period, the cheese vat must not be agitated or disturbed in any way, or a soft or weak curd will result and yield will be affected.

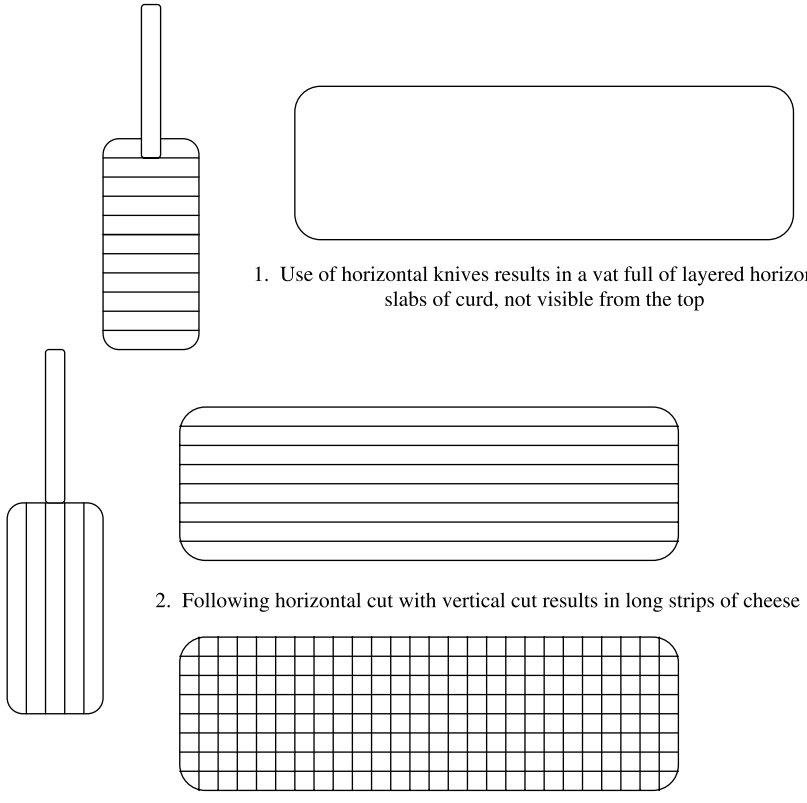
Chymosin is allowed to set the cheese for 20–30 min prior to curd testing. In large automated plants, the curd is typically not checked, and cutting begins at a set time. To check the curd, a spatula or knife may be used. A spatula works best for checking curd set because of its rounded shape. The blade is cut through the curd in a 5-cm vertical orientation and removed. The blade is then inserted at the bottom of the vertical cut, in a horizontal orientation, to form a T. The blade is pushed forward and lifted, to encourage the curd to split open. The curd is ready for cutting when the curd is firm, it breaks cleanly, and fills with clear yellow (not cloudy) whey.

24.3.3.6 Cutting. Cutting of the curd is an extremely important step in the cheesemaking process because it influences whey drainage and cheese yield (Ramet 2000a). Cutting to a consistent size, with sharp knives, is critical to minimize small curd particles (fines) that may get lost during whey drainage (Ramet 2000a). Manually, the coagulated mass of cheese is cut with harps: knives constructed of stainless steel hardware and wire spaced at regular intervals. The wires on one harp are horizontally oriented, and the other harp wires are vertically oriented. The cutting progresses in such a way that first horizontal and vertical sheets of curd are cut with the harp knives. The sheets are then cut into cubes by perpendicular cuts with the vertical harp knives (Fig. 24.2).

Large dairy plants have automated cheese vats that vary in size from 2000 to 25,000 L capacities. These cheese vats are equipped with a shaft to which agitators are attached. These agitators are designed in such a way that they cut the cheese when the shaft rotates in one direction and agitate the cubes gently when the shaft rotates in the other direction. Cheese vats are automated and allow the cheese curds to heal and cook before pumping the curds and whey onto a perforated conveyer belt where cheese curd is separated from the whey.

A schematic diagram of cheesemaking from cutting through cooking steps is shown in Figure 24.3. Cutting the coagulum increases the surface area of the curd and enhances syneresis. Upon cutting, curd particles immediately begin to expel whey and shrink, and the TA that had been rising in the cheese milk immediately drops in the whey, because the whey has lower apparent acidity, due to lower protein, citrate, and phosphate. The TA of whey will gradually increase as lactic acid is formed in the curds and released with whey during syneresis.

24.3.3.7 Healing. Freshly cut curd is fragile and shatters easily, so curds are allowed to "heal" for 5–10 min prior to agitating and cooking. A healing period is particularly important when goat Cheddar cheeses are made, because the curds are naturally more fragile than curd obtained from cow milk. During healing, a tender skin is formed



1. Use of horizontal knives results in a vat full of layered horizontal slabs of curd, not visible from the top

2. Following horizontal cut with vertical cut results in long strips of cheese

3. Only after the vertical sharp knife is used perpendicularly across the previous cuts will cubes be made out of the curds, enabling optimal syneresis

Figure 24.2 *Cutting of the curd.*

around each freshly cut curd. As the skin firms, the curd becomes more resistant to shattering and yield losses.

24.3.3.8 Cooking. The cooking process is essentially a controlled increase in curd–whey temperature. Heating allows individual curd cubes to shrink, release whey, and firm. Cooking also increases reaction rates, specifically bacteria growth and metabolism, and enzyme activity. Temperature-sensitive bacteria strains are slowed down as temperature is raised. Prior to raising the temperature of the curd–whey mixture, curds should be gently eased from the edges of the cheese vat, where they have matted. Curd cooking should begin slowly, with continual stirring of the curds. Hot water or steam may be used to increase the jacket temperature. The curd–whey mixture temperature should be raised slowly, about 2°C every 5 min until 38°C is reached (~35–45 min). Stirring speed may be increased as the curds firm, but stirring too fast will shatter curds and reduce yield. For a drier cheese, temperature should be held at 38°C for an additional 45 min, with stirring. In small or start-up facilities, whey TA should be recorded every 15 min. Regardless of plant size, good records should be kept of the entire cheesemaking procedure and final cheese quality. Failure to keep such records will reduce consistency.

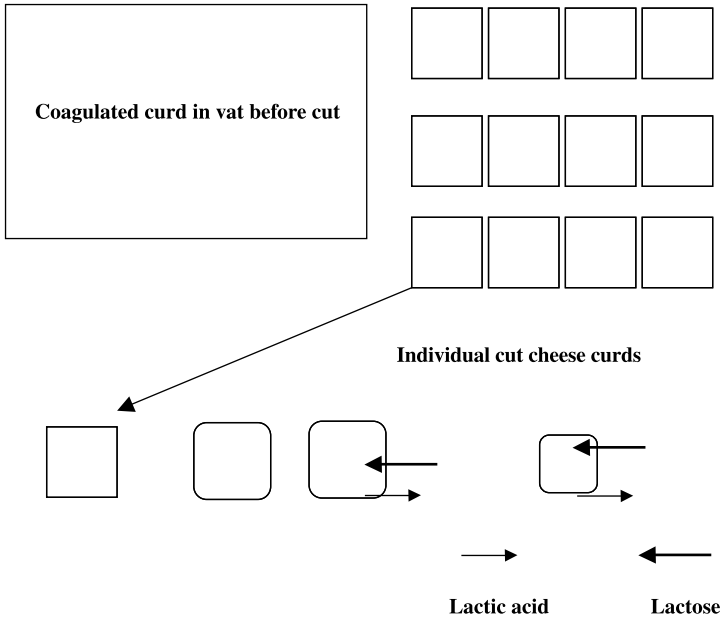


Figure 24.3 *Schematic of cheesemaking steps from cutting through cooking.*

For a short period after the curd is cut, lactose and lactic acid concentrations are at equilibrium in curd and whey. With time, the concentration of lactose drops faster in the curd than in the whey because starter bacteria concentrated in the curd deplete lactose in the curd (Fig. 24.3). As the lactose is fermented within the curd, replacement lactose diffuses into the curd from the whey (Fig. 24.3, broad arrows) (Lawrence and Gilles 1987a). As a neutral molecule, lactose diffuses easily through the matrix. Positively charged hydrogen ions exit the negatively charged curd much more slowly than lactose (Fig. 24.3, narrow arrows). As fermentation progresses, hydrogen ions are neutralized by the negatively charged proteins and phosphates. As the buffering capacity of the caseins and colloidal calcium phosphate (CCP) becomes saturated, the pH of the curd steadily drops. As pH drops, CCP is solubilized and lost into the whey.

Once cheesemaking starts, managing acid development during the cooking stage of cheese manufacture is the most important factor in the control of cheese quality (Lawrence and Gilles 1987a). Acid development determines the basic structure, moisture, final pH, and flavor of cheese (Lawrence and Gilles 1987a). As the pH drops, the body of the cheese changes from rubbery (pH 5.4) to plastic (pH 5.3–5.2) to Cheddar (pH 5.1–5.0) body and texture. When the vat TA rises too quickly, the curd will suffer an excessive loss of calcium but will not retain phosphate. The result is an increase in curd buffering capacity (Lawrence and Gilles 1987a). However, should a high TA result from an extended time between cutting and cheddaring, both calcium and phosphorus are lost in the whey (Lawrence and Gilles 1987a). The resultant cheese will have a low pH, an acid flavor, and a weak, pasty texture (Lawrence and Gilles 1987a). An objective for cheesemakers it to develop acid slowly during ripening and cooking, and more quickly during cheddaring, so that calcium phosphate is retained in the curd, as the loss of CCP alters the body and texture of the cheese (Lawrence and Gilles 1987a). Curds shrink and tighten as syneresis proceeds during the cooking process, prior to whey drainage. Approximately

75% of the whey in the curd is expelled in the time from cutting to the end of stir out. Longer stir-out times can significantly reduce moisture in cheese.

24.3.3.9 Draining. Whey may be drained entirely, in the case of Cheddar, or partially, with washing, as in the case of Colby or Monterey. In large plants, a cleaned and sanitized finishing table/vat may be aligned with the exit port of the cheese vat. The drain of the vat is opened and curds and whey are allowed to flow onto the finishing table. Alternatively, in small plants, the cheese vat may double as a finishing table. A screen is installed ahead of the finishing table drain port to prevent curd loss as the whey is drained. Whey is commonly collected in a separate reservoir.

Cheese curds should be allowed to settle into the vat or finishing table, at an even depth throughout the length, and permitted to mat for 15 min. Whey TA will rise more quickly during this interval and should be recorded every 15 min from this point forward, throughout the cheddaring process. In the largest plants, curds are delivered to a perforated conveyer belt for drainage and cheddaring, which allows formation of a sheet of curd and continuous whey drainage. The conveyer is enclosed in a tunnel. Upon drainage, in the absence of the whey bath, the curd pH will drop at a faster rate and the curds will continue to shrink and tighten. Much of the calcium is lost at drainage, particularly at low pH.

24.3.3.10 Washing. Washing is essential in Colby and Monterey production. Washing, or curd rinsing, removes lactic acid and residual lactose and lactic acid from the curd and the result is a higher pH in the final cheese. Washing is rarely included during Cheddar cheesemaking, but when it is, the duration of such rinsing is so limited that only the whey on the surface of the curds is removed (FDA 2004c).

In Colby and Monterey production, whey is drained off until the curd on the bottom of the vat is visible, then sufficient cold water is introduced to reduce the temperature of the curd–whey mixture to 27°C (Lawrence and Gilles 1987a). Rate of syneresis is slowed if cool water used, resulting in higher cheese moisture content. Long wash time removes more lactose, resulting in higher final pH of cheese. Temperature-sensitive strains may be revived if cool water used.

24.3.3.11 Cheddaring. The step known as "cheddaring" was standardized into commercial practice by Joseph Harding in 1857 (Kosikowski and Mistry 1997a). During manual cheddaring, curds are flipped and stacked at regular intervals, naturally pressed under their own weight, which enhances syneresis, yet still maintains a controllable level of moisture retention. The main purpose of cheddaring is to allow time for the acidity to increase and whey to be released (Lawrence and Gilles 1987a). Curd particles fuse into a solid mass, syneresis continues as acid builds, rennet/chymosin continues to act, and these forces cooperate to tighten the casein network. As lactic acid continues to build, curds begin to flow or stretch under the weight of piled slabs. Cheddar gains its characteristic body through the process of knitting, stretching, and orientation of the casein network during cheddaring, which requires a pH below 5.8 (Lawrence and Gilles 1987a).

In manual operations, after the cooking step is completed and curds are allowed to settle for 15 min, matted curd should be trenched, then cut into equal-sized slabs. A wide (20–30-cm) trench is made in the center of the vat to facilitate syneresis and curd stretching. Slabs should be separated as they are cut, to enable syneresis and stretching. Extra or broken curd should be placed on top of each slab, to minimize loss of fines. Slabs should be allowed to settle 15 min before the next step.

Cheddaring begins with flipping of slabs, one by one. The bottom becomes the top and the end toward the trench becomes the end toward the vat wall. Slabs should be allowed to settle 15 min before each subsequent step. The next step of cheddaring involves the flipping of one slab, followed by placing of an adjacent slab on top of the flipped slab (without flipping). This step is called "flip–stay." The process continues for every pair of slabs. After 15 min, the top slab is placed (not flipped) into an empty spot in the vat. The previous bottom slab is then flipped and placed atop the new bottom slab. This step is called "stay–flip." Cheddaring continues with flipping and stacking of slabs, alternating between "flip–stay" and "stay–flip" steps, until a whey TA of 0.35% as lactic acid is measured in a fresh sample of whey. In large plants, the process of cheddaring is automated. As the perforated conveyor mentioned previously transects a number of parallel planes during the approximately 90-min cheddaring process, the matted curds are flipped and stretched continuously in a tumbling motion.

24.3.3.12 Milling. Milling is the process of cutting the slabs into cubes about 5 cm in size, which enables more uniform salt distribution, encourages syneresis and makes hooping more convenient (Lawrence and Gilles 1987a). When curds are milled, more whey is expelled because milling greatly increases curd surface area and opens pores for syneresis. Salt distribution will be most uniform in cheese if curds are milled to a uniform size (Lawrence and Gilles 1987a). In large plants, as the mat of curd arrives at the discharge point of the perforated conveyer, it is cut to desired size in a reciprocal dice-type mill or rotary curd mill.

24.3.3.13 Salting. Milled curds of Cheddar and related hard cheeses are dry-salted rather than brine-salted. Cheese is salted because it:

1. Encourages further syneresis,
2. Inhibits further growth and metabolism of most microorganisms (thus arresting lactic acid production), and
3. Provides flavor.

Approximately 2.5 kg salt for every 100 kg of cheese curd is used. The salt is added in three equal applications and mixed for 5 min between applications. Adding salt too quickly will cause a "skin" to form on the curds, inhibiting salt absorption and syneresis. In large plants the milled cheese quantity is determined continuously by weight prior to entering the salting machine. The salter automatically calculates the salt and sifts it over the milled cheese. The pH and TA will only change slightly beyond the point of salt addition.

Salt, more specifically salt in moisture (S/M), directly influences the final pH of the cheese, growth of microganisms and overall flavor, body, and texture of cheese (Lawrence and Gilles 1987a). At S/M levels greater than 5.0, bitter flavors rarely occur (Lawrence and Gilles 1987a). Curd salted at low TA retains more salt (higher S/M) and is more plastic than curd salted at high TA (Lawrence and Gilles 1987a).

24.3.3.14 Pressing and Packaging. Pressing gives cheese its final shape, reduces openings between curd particles, promotes fusion, and releases more free whey. In small plants, Cheddar curds are pressed overnight using a batch method (Lawrence and Gilles 1987a). Pressure, approximately 1.4 atm, is applied to molds for 8–12 h at room

temperature. After one or two hours of pressing, cheeses may be flipped in the molds and lined with cheesecloth, which provides an attractive surface pattern. Large plants have a continuous "block-former" system (Lawrence and Gilles 1987a). Curds are fed into a tower under a partial vacuum, whey is siphoned off, and for a short period, mechanical pressure is applied at the base of the tower prior to packaging (Lawrence and Gilles 1987a; Banks and Williams 2004). A block former cuts 20-kg blocks from the stack at regular intervals and the blocks are transferred to a vacuum packaging system prior to aging (Banks and Williams 2004).

24.3.3.15 Aging. Aging enables flavor and texture development of hard cheeses. Nearly all residual lactose should be fermented within about 48 h. With cold storage, between 5 and 12°C, acid production slows down, but continues until limiting conditions occur (Banks and Williams 2004). Starter bacteria lyse (burst) and release proteolytic enzymes into the matrix. Residual plasmin and coagulant also contribute to proteolysis during aging. Caseins are broken down into peptides and amino acids, which yield flavor and modify cheese body/texture. Secondary fermentations can occur if NSLAB are still active, which results in further changes in flavor and body/texture. Cheeses with low S/M have a higher rate of proteolysis, resulting in a softer texture, than cheeses with high S/M (Lawrence and Gilles 1987a).

Cheddar cheese is typically aged for 3–18 months at 7–13°C, but it is not unheard of to age Cheddar for years in the case of specialty varieties (Banks and Williams 2004). Colby and Monterey are aged for shorter periods of time due to their higher moisture content and milder expected flavor.

24.4 QUALITY CONTROL

24.4.1 Shelf-life

The shelf-life of Cheddar and related cheeses is limited by quality, not safety. The quality of good Cheddar cheese improves with storage. Cheddar cheese may be removed from shelves due to flavor, body, or appearance defects. The most common flavor defects are high acid, bitter, unclean, and fermented/fruity. Common body defects are weak or crumbly body, gas holes, surface discoloration, and appearance of crystals on surfaces.

24.4.2 Evaluation

High-quality Cheddar cheese has a full, balanced nutty, sharp, but not bitter flavor. The ideal texture should be closed (no gas holes or mechanical openings), and the body should be firm, smooth and waxy (responds to moderate pressure). Colby and Monterey/Jack cheeses are similar to Cheddar, but are milder in flavor and possess a softer body. Colby and Monterey/Jack are prone to the same defects as Cheddar. However, due to higher moisture content, lower acid and salt, and higher microbial and enzymatic activity, some sensory defects may reach greater intensity and frequency in Colby and Monterey/Jack cheeses than Cheddar, particularly with extended aging.

Gas liquid chromatography (GLC) analysis of Cheddar cheese has shown that there are as many as 200 different compounds that may contribute to cheese flavor. However, flavor chemists believe that as few as 20 volatile compounds are pertinent to determination of the eventual flavor of Cheddar cheese. Cow diet, milk handling and sanitation

TABLE 24.2 Common Flavor Attributes in Hard Cheeses, Identification of Them and Their Probable Causes.

Flavor	Identification	Probable Cause
Bitter	Sometimes perceived as throbbing/piercing Sensation perceived at back of tongue Very common defect in aged Cheddar	Excessive moisture Low salt Excessive acidity Proteolytic starter culture strains Microbial contaminants Poor quality milk Plant sanitation issues
Feed	Grassy flavor	Feeding of strong flavored feeds Feeding of cattle too close to milking
Fruity/fermented	Sweet – like pineapple	Psychrotrophic *Pseudomonas fragi* may produce ethyl-butyrate and ethylhexanoate (esters) Low acidity Excessive moisture Low salt level Poor milk quality
Flat/lacks flavor	Lacks nutty flavor components Lacks typical Cheddar flavor	Lack of acid production Use of milk low in fat Excessively high cooking temperature Use of low curing temperature Too short a curing period
Heated	Sweet – like cooked flavor Reminiscent of Velveeta®	High pasteurization or cooking temperature
High acid	Excessive acid taste Unbalanced acid taste	Development of excessive lactic acid Excessive moisture Use of too much starter culture Use of high-acid milk Improper whey expulsion from curd Low salt level
Oxidized	Paperboard/cardboard Sometimes discoloration also Burnt hair aroma/flavor	Use of oxidized milk in cheesemaking Excessive exposure to UV light during aging
Rancid	Butyric, caproic, caprylic, capric acids Soapy Romano cheese aroma/flavor Baby vomit aroma	Milk lipase activity Microbial lipase activity (from contaminants) Accidental homogenization of raw milk Late lactation or mastitic milk
Sulfide	Eggy	Excessive breakdown of amino acids Only a defect when excessive for age of cheese
Unclean	Unpleasant off-flavor lingers	Microbial contamination Poor quality off-flavored or old milk Allowing off-flavored cheese to be aged Improper techniques of cheddaring
Whey taint	Combination of acid, bitter, fermented Aftertaste does not linger like unclean	Poor whey expulsion from curd Improper cheddaring techniques Failure to drain whey from piles of curd slabs
Yeasty	Ethanol aroma Yeast (bread, beer) aroma	Development of ethanol flavors by yeast contaminants Poor packaging procedures

TABLE 24.3 Common Appearance, Body and Texture Attributes in Hard Cheeses, Identification, and Their Probable Causes.

Body/Texture	Identification	Probable Cause
Corky	Dry, noncompressible Often crumbly as well	Lack of acid development Low fat
Crumbly	Falls apart while working	Excessive acid production Low moisture retention in cheese
Crystals	White crystals observed by visual examination	Tyrosine (only in aged cheese), calcium lactate, calcium citrate, calcium phosphate
Curdy	Resistant to compression	Inadequate aging conditions
Gassy	Smooth round gas holes	Contamination of cheese with CO_2-forming microorganisms
Mealy	Grainy (like corn meal)	Excessive acid production Formation of salt complexes
Open	Openings along curd lines	Improper mechanical pressing, lack of fusion between curds
Pasty	Sticky when working between fingers	High moisture retained by curd Excessive acid production
Short	Plug breaks quickly (snaps)	Excessive acid production
Weak	Plug is resistant to breaking (bends)	High moisture in cheese Excessive proteolysis

practices, milk composition, and cheese manufacturing conditions all affect cheese chemistry (Buchin and others 1998). What appears to be critical is the relative proportions of the key chemical flavor compounds in providing "balanced Cheddar flavor". Ammonia-like and sulfur-like odors and bitter taste typically occur in aged cheeses, a consequence of amino acid breakdown. Common flavor attributes encountered in Cheddar and related cheeses are included in Table 24.2. Although some attributes, such as sulfide, may be considered desirable in an aged cheese, mild cheeses are discredited for pronounced attributes.

In addition to flavor attributes, consumers look for certain functional properties in cheese (melting, grinding, slicing). Cheeses continually change during ripening, not only in flavor, but also in body/texture. Common body/texture attributes encountered in Cheddar and related cheeses are included in Table 24.3.

24.4.3 Safety

Cheddar cheese and other semihard cheeses are generally considered safe and have rarely been associated with foodborne illness outbreaks (Wood and others 1984; Johnson and others 1990; El-Gazzar and Marth 1992; Leyer and Johnson 1992). However, some pathogens can survive the cheesemaking process and during ripening. Hargrove and others (1969) demonstrated that cheese pH, rate and amount of acid produced during cheesemaking, and type and amount of starter inoculum all influence growth and survival of salmonellae in Colby and Cheddar cheeses. Raw-milk Cheddar cheese was linked to major salmonellosis outbreaks in Canada in 1982, 1984, and 1998 (Wood and others

1984; D'Aoust and others 1985; El-Gazzar and Marth 1992; Ratnam and others 1999). *Salmonella* can survive in ripening Cheddar cheese for 7–10 months (Wood and others 1984; El-Gazzar and Marth 1992). Additionally, Ryser and Marth (1969) showed that *L. monocytogenes* can survive as long as 434 days in Cheddar cheese ripened at temperatures above 1.66°C. These facts highlight the importance of using the highest quality milk and good manufacturing practices, including strict sanitation and handling procedures to prevent contamination and ensure cheese quality and safety.

24.5 TROUBLE-SHOOTING

Although poor-quality milk will always result in poor-quality cheese, even use of high-quality milk does not guarantee high-quality cheese. This section will summarize factors that influence cheese curd formation and crystal formation in Cheddar cheese.

24.5.1 No Curd or Weak Curd Formation

A weak curd can result from at least one of two main factors, namely low starter numbers or poor chymosin activity. The following factors influence the starter and chymosin activity: presence of natural inhibitors or antibiotics in the milk, residual cleaners/sanitizers on equipment, or the presence of bacteriophage in cultures or in the environment. Each factor will be discussed separately. Operator error is another reason for no curd formation. The bottom line is that personnel must be adequately trained to (1) measure appropriate levels of culture and coagulating enzyme to be added to cheese milk and (2) actually add the ingredients to the cheese milk.

24.5.2 Natural Inhibitors

Natural inhibitors in milk include lactenin L_1, L_2, and L_3, and the enzymes lysozyme and lactoperoxidase. Lactenin varies with individual animals and is inactivated by heat treatment (Desmazeaud 2000). Lysozyme attacks the glycosidic bonds found in Gram-positive bacterial cell walls, but it is unlikely to cause inhibition of lactic acid starter bacteria (Jensen 1995; Desmazeaud 2000). When supplied with hydrogen peroxide (from lactic acid bacteria in the presence of oxygen) and thiocyanate (arises from catalysis of thiosulfate or glucosides in liver), lactoperoxidase will catalyze the formation of lactococci bacteriocides (Ruden 1997), including bacteriocides that attack lactococci. Therefore, this method of preserving milk in areas with limited access to refrigeration is inappropriate for handling cheese milk. Mastitic milk naturally contains higher levels of leucocytes, which will also engulf and destroy lactic cultures (U.S. Department of Health and Human Services and others 1999 Revision).

24.5.3 Antibiotics

Every tanker load of milk must test negative for the presence of beta-lactam antibiotics (Desmazeaud 2000), however cultures can be inhibited by the presence of antibiotics at levels even lower than detectable by standard dairy lab testing methods. Lactic cultures can be inhibited by as much as 50% by 1.91 μg cloxacillin, 0.13 μg tetracycline,

0.59 μg streptomycin and as little as 0.12 μg of penicillin per mL milk (Desmazeaud 2000). Thermophilic bacteria are more resistant to streptomycin and more sensitive to penicillin than mesophilic starters (Ruden 1997).

24.5.4 Residual Cleansers/Sanitizers

Residual cleansers/sanitizers can slow a cheese vat precisely because they are intended to kill microorganisms. Quaternary ammonium compounds, or "Quats" are not appropriate for use in a cheese plant because they leave a residue on equipment that are effective against lactic acid bacteria. Quats will inhibit many starter culture strains at concentrations as low as 10 to 20 μg/mL (Ruden 1997). Other effective bactericides include organic and inorganic chlorine compounds, chlorine dioxide, iodine compounds, acid anionic sanitizers and peroxyacetic acid, but they are unstable in the presence of organic matter such as milk (Leach 1997). Regardless of sanitizer, pipelines, vats and other equipment must be allowed to drain after bactericidal treatment to prevent contamination of the cheese milk supply.

24.5.5 Bacteriophage

Bacteriophage literally means "eaters of bacteria." Bacteriophage/phage are obligate intracellular parasites that attack and replicate within specific strains of bacterial cells (Leach 1997). Bacteriophage are naturally present in the cheesemaking environment and can spread throughout a plant with poor sanitation practices. Bacteriophage are the largest single cause of slowed or failed vats of cheese (Tamime and Deeth 1980; Leach 1997). Each strain of culture has a different level of sensitivity to bacteriophage. Since it is impossible to entirely eliminate bacteriophage from a dairy plant operation, control measures must be employed. Careful selection of starter cultures, aseptic techniques for starter culture propagation, air filtration, equipment sterilization, plant sanitation, culture rotation of phage-unrelated strains, or use of phage-resistant strains are necessary techniques to control phage (Leach 1997). Starter culture rotation essentially ensures that the bacteriophage population is diluted (through the process of repeated sanitation efforts) to the point that their numbers are low enough to allow the starter culture to function normally (Desmazeaud 2000).

24.5.6 Milk Pretreatment

Excessive heating, agitation or aeration can reduce growth and acid development by lactic acid bacteria. Heating of milk to pasteurization only slightly modifies the characteristics of milk for cultures. However, temperatures above 80°C for 20 sec. induce chemical reactions that can either inhibit (by destruction of certain vitamins) or stimulate (destruction of lactoperoxidase, production of formic acid from lactose, release of non-protein-nitrogen) bacterial growth (Ruden 1997). Excessive heating of milk should be avoided, not simply because of subsequent effects on culture, but the detrimental effects on curd formation, curd moisture retention and cheese quality. Aeration or excessive agitation will slow the vat by introducing dissolved oxygen. Presence of oxygen inhibits starters since they prefer a micro-aerophilic environment (McDowall and McDowell 1939).

24.6 CRYSTAL FORMATION

The occurrence of undesirable crystals in Cheddar cheese has been documented since the 1930s (Tuckey and others 1938; McDowall and McDowell, 1939; Harper and others 1953; Conochie and others 1960; Pearce and others 1973; Washam and others 1985; Severn and others 1986), yet the problem still represents a challenge and expense to cheese manufacturers (Chou and others 2003). Cheese crystals have been identified as calcium lactate (Severn and others 1986), a racemic mixture of L(+)- and D(−)-calcium lactate (Conochie and Sutherland 1965), calcium phosphate (Dorn and Dahlberg 1942; Harper and others 1953; Conochie and others 1960), tyrosine (Harper and others 1953; Bianchi and others 1974), or mixtures of amino acids (Severn and others 1986; Dybing and others 1988; Johnson and others 1990a,b). However, most frequently the crystals in young cheese have been identified as calcium lactate (Pearce and others 1973; Blake and others 2005).

The development of CLC may result from a number of causes, including milk composition (Dybing and others 1988), cheesemaking procedure (Pearce and others 1973; Dybing and others 1988; Johnson and others 1990b; Chou and others 2003), aging temperature (Johnson and others 1990b; Somers and others 2001; Chou and others 2003), and the growth of nonstarter lactic acid bacteria (NSLAB) in cheese during aging (Dybing and others 1988).

Cheese milk lactose concentrations exceeding 4.8% lead to increased lactose in cheese, which can be used by starter or NSLAB to produce lactate in cheese and a resultant increase in calcium lactate concentrations. Dybing and colleagues (1988) concluded that casein-bound calcium is the major source of calcium in calcium lactate crystals (CLC). The investigators showed that fast acid production and high milling acidities are associated with reduced CLC formation owing to reduced concentrations of casein-bound calcium. Seasonal changes affecting milk casein and calcium affect CLC formation. A low casein to calcium ratio leads to increased amount of bound calcium in milk, contributing to increased calcium in cheese and greater predisposition to CLC formation (Khalid and Marth 1990; Williams and others 2000; Agarwal and others 2006a).

Although starter bacteria make up the majority of cheese microflora initially, NSLAB dominate the viable population in cheese for much of the ripening period (Somers and others 2001). Secondary NSLAB introduced at the cheese plant (Blake and others 2005) or during cut and wrap, may proliferate upon stimulation by warm tempering temperatures (Dybing and others 1988; Williams and others 2000). Heterofermentative NSLAB utilize a variety of substrates for growth and produce an assortment of metabolites, including both L(+)- and D(−)-lactate (Johnson and others 1990b). NSLAB that are capable of racemizing L(+)- to D(−)-lactate can contribute to CLC since D(−)-lactate is less soluble than L(+)-lactate, particularly at aging temperatures, in maturing cheese (1990b). In the early 1990s, Johnson and colleagues (Turner and Thomas, 1980; Shakeel Ur and others 2000) established correlations among CLC, D(−)-lactic acid enantiomer, and numbers of NSLAB. Cheeses with racemase-positive *Lactobacillus* developed crystals compared to cheeses without *Lactobacillus*, which did not. Cheeses aged at lower temperatures developed crystals faster when compared to cheeses aged at higher temperature. CLC were never observed on cheeses with less than 20% of the lactic acid in the D(−) form. It follows, then, that since high temperatures favor the growth of NSLAB (Johnson and others 1990b), aging cheese at high temperatures, as may be done to accelerate ripening, can result in elevated D(−)-lactate by NSLAB and induction of CLC (Linke 1958).

Additionally, aging of cheese at low temperatures may also increase CLC, due to decreased solubility of calcium lactate at a low temperature (Chou and others 2003). Chou and colleagues (2003) demonstrated that earliest and most extensive CLC occurred on cheeses aged first at higher temperatures and then stored at lower temperature. They also showed that specific NSLAB, production of D(−)-lactate, and aging temperature affect CLC in maturing Cheddar cheese (Chou and others 2003).

Elimination of racemizing NSLAB and control of storage temperature is critical to the prevention of CLC. Agarwal (2006a) and Sharma (2002) showed that, irrespective of lactose to protein ratio, contamination of cheese milk with racemizing NSLAB *L. curvatus*, may lead to CLC, particularly in combination with elevated storage temperatures. Finally, Agarwal and colleagues (2005) demonstrated that, regardless of the presence of racemizing NSLAB, CLC are more likely to form in cheeses flushed with gas than cheeses that are vacuum packaged.

More recent research (Agarwal and others 2006b) shows cheese milk composition and cheese making techniques have greater influence on the occurrence of CLC, particularly calcium L(+) lactate crystals. Increased casein concentration in cheese milk is linked to increased colloidal calcium, which solubilizes as the pH of the cheese decreases (Upreti and others 2006). If cheese is made such that it has increased residual lactose, microorganisms present in cheese will ferment residual lactose to produce excess lactic acid and decrease the pH of the cheese, encouraging solubilization of colloidal calcium previously bound to casein micelles. Increases in concentrations of soluble calcium and lactate above saturation in cheese serum during aging tends to favor development of CLC. Control of pH and whey removal prior to packaging influence the occurrence of CLC.

In summary, cleaning, sanitizing, prevention of contamination of cheese milk with lactate-racemizing NSLAB, acidification and whey removal, consistent storage temperature and vacuum packaging are encouraged to minimize CLC. Due to the prevalence and expense of the problem in the industry, additional research in the area of CLC formation is warranted.

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25

Pasteurized Process and Related Cheeses

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25.1 Introduction 596
25.2 Definitions 596
25.2.1 Pasteurized Process Cheese 598
25.2.2 Pasteurized Process Cheese Food 598
25.2.3 Pasteurized Process Cheese Spread 599
25.2.4 Pasteurized Process Cheese Product 599
25.2.5 Cold-Pack Cheese 599
25.2.6 Cold-Pack Cheese Food 599
25.2.7 Cold-Pack Cheese Spread 600
25.3 Principles of Processing 600
25.3.1 Ingredients 600
25.3.1.1 Cheeses 600
25.3.1.2 Emulsifying Agents 601
25.3.1.3 Optional Ingredients 603
25.3.2 Production 605
25.3.2.1 Selection and Analysis 605
25.3.2.2 Preparation 606
25.3.2.3 Mixing 607
25.3.2.4 Cooking 607
25.3.2.5 Homogenization 609
25.3.2.6 Packaging and Cooling 609
25.4 Product Attributes 610
25.4.1 Nutrition 610

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

25.4.2 Quality 611
25.4.3 Shelf-Life and Safety 611
25.5 Summary/Conclusions 614
References 614

25.1 INTRODUCTION

Approximately one-third of the cheese produced in the United States is marketed as pasteurized process cheese (Kosikowski and Mistry 1997). Why? Fast-food giants like McDonald's and Burger King rely on the consistency in flavor and melting qualities of pasteurized process cheese. Pasteurized process cheese, sometimes called processed cheese or process cheese, is convenient not only for food service, the primary user, but also retail because these cheeses are available in regular and reduced-fat varieties, chunks, cubes, spreads, loafs, slices, as well as in grated or shredded applications.

American process cheese was first developed as early as 1895 (Caric and others 1985; Kosikowski and Mistry 1997). In 1905, J.L. Kraft was making money by selling cheese from a horse-drawn delivery wagon (Kosikowski and Mistry 1997). Eleven years later, Kraft was issued a patent involving the heating of Cheddar cheese and its emulsification with alkaline salts, inaugurating American pasteurized process cheese (Kosikowski and Mistry 1997). In 1911, Swiss inventors preserved Emmental cheese by applying heat and adding emulsifying agents to form a smooth and uniform flowing mass that solidified upon cooling (Berger and others 1989). As is true today, pasteurized process cheese was initially manufactured to utilize natural cheeses that would otherwise be unmarketable, including cheese with mechanical openings, deformations, localized molds, and trimmings produced during cheese formation pressing and packaging (Caric and others 1985; Caric and Kaláb 1999).

Today, approximately 635 million kg of pasteurized process cheeses, 407 million kg of pasteurized process cheese foods and spreads, and 12 million kg of cold-pack cheese and cheese foods are produced in the United States each year, by more than 50 processing plants (IDFA 2002; Infanger 2004). Converted to per capita consumption, Americans averaged 1.9 kg pasteurized process cheese and 1.6 kg pasteurized process cheese food and spreads in 2001 (IDFA 2002). This chapter will elaborate upon similarities and differences among pasteurized process and cold-pack cheeses, outline manufacturing steps involved in production, and describe technological advances in the production of cheeses in these categories.

25.2 DEFINITIONS

The Code of Federal Regulations (CFR) defines more than 12 different kinds of pasteurized process and cold-pack cheeses, including:

- Cold-pack and club cheese;
- Cold-pack cheese food;
- Cold-pack cheese food with fruits, vegetables, or meats;
- Grated American cheese food;

- Pasteurized blended cheese;
- Pasteurized blended cheese with fruits, vegetables, or meats;
- Pasteurized process cheese;
- Pasteurized process cheese with fruits, vegetables, or meats;
- Pasteurized process pimento cheese;
- Pasteurized process cheese food;
- Pasteurized process cheese food with fruits, vegetables, or meats;
- Pasteurized cheese spread;
- Pasteurized cheese spread with fruits, vegetables, or meats;
- Pasteurized process cheese spread; and
- Pasteurized process cheese spread with fruits, vegetables, or meats (FDA 2004a).

Although different, all of the products have one thing in common: they must contain a minimum percent of natural cheese and a maximum percent of water in the composition. Characteristics of the main categories of pasteurized process and cold-pack cheeses are summarized in Table 25.1.

TABLE 25.1 Characteristics of Pasteurized Process and Cold-Pack Cheeses.

Type	Ingredients	Cooking Temperature	Composition	pH
Pasteurized process cheese	Natural cheese, water, anhydrous milk fat, cream, color, salt, flavoring, and emulsifiers	70–80°C	Moisture and fat correspond to legal limit of natural cheese	5.6–5.8
Pasteurized process cheese food	Same as process cheese, optional ingredients: skim milk, cream, whey, organic acids	79–85°C	Not $>$44% H_2O or $<$23% fat, but $>$22% fat if vegetables, fruits, or meats included	5.2–5.6
Pasteurized process cheese spread	Same as cheese food but also gums are added for water retention	88–91°C	Not $<$44% or $>$60% H_2O; not $<$20% fat; spreadable at 21°C	$\leq$5.2
Cold-pack cheese	Same as process cheese, except cheese ingredients are pasteurized or aged	Not heated	Same as natural cheese: $\leq$42% H_2O, $\geq$47% fat	$>$4.5
Cold-pack cheese food	Same as process cheese food, but allow gums, sweetening agents, inclusions; cheese must be aged or made from pasteurized milk	Not heated	$\leq$44% H_2O, $\geq$23% fat	$>$4.5

Source: CFR. Requirements for specific standardized cheese and related products. Subpart B vol. 133.

25.2.1 Pasteurized Process Cheese

The Code of Federal Regulations (FDA 2004d) defines pasteurized process cheese (PC) as "the food prepared by comminuting and mixing, with the aid of heat, one or more cheeses of the same or two or more varieties . . . for manufacturing with an emulsifying agent . . . into a homogeneous plastic mass". The CFR excludes cream cheese, Neufchatel cheese, cottage cheese, low-fat cottage cheese, cottage cheese dry curd, cook cheese, hard grating cheese, semisoft part-skim cheese, part-skim spiced cheese, and skim milk cheese from the list of permissible cheeses in PC.

Moisture content of PC is defined by whether the PC is made from one or more types of cheeses. In cases where a single variety of cheese is used, the moisture content of a PC is not more than 1% greater than the maximum moisture content prescribed by the definition and standard of identity, if any, for the variety of cheese used, but in no case is more than 43%. However, the moisture content of pasteurized process washed curd cheese or pasteurized process Colby cheese must not exceed 40%; the moisture content of pasteurized process Swiss cheese or pasteurized process Gruyere cheese must not exceed 44%; and the moisture content of pasteurized process Limburger cheese must not exceed 51% (FDA 2004d). When made from two or more varieties of cheese, the moisture content of a PC is not more than 1% greater than the arithmetical average of the maximum moisture contents prescribed by the definitions and standards of identity, if any, for the varieties of cheese used; but in no case is the moisture content more than 43%. However, the moisture content of a PC made from two or more varieties Cheddar cheese, washed curd cheese, Colby cheese, and granular cheese must not exceed 40%, and the moisture content of a mixture of Swiss cheese and Gruyere cheese must not exceed 44% (FDA 2004d).

Similarly, PC fat content is limited by the cheese(s) that compose the PC. If a single cheese variety is used, the fat content of the solids of a PC is not less than the minimum prescribed by the definition and standard of identity, if any, for the variety of cheese used, but in no case is less than 47%. However, the fat in dry matter of pasteurized process Swiss cheese must not be less than 43%, and the fat in dry matter of pasteurized process Gruyere cheese must not be less than 45%. When two or more varieties of cheese are combined, the fat content of the solids of a PC is not less than the arithmetical average of the minimum fat contents prescribed by the definitions and standards of identity, if any, for the varieties of cheese used, but in no case is less than 47%. However, the fat content of the solids of a pasteurized process Gruyere cheese made from a mixture of Swiss cheese and Gruyere cheese must not be less than 45%.

Process cheese may be smoked, or the cheese or cheeses from which it is made may be smoked, before mixing, or it may contain substances prepared by condensing or precipitating wood smoke (FDA 2004d).

25.2.2 Pasteurized Process Cheese Food

The difference between PC and pasteurized process cheese food (PCF) is primarily compositional, but the body of PCF may be notably softer and the flavor milder. PCF is allowed more water and less fat; cooking temperatures are higher and the pH is lower, and additional ingredients are allowed. Additional ingredients include skim milk, milk, cream, cheese whey, buttermilk, albumin from cheese whey, and skim milk cheese (FDA 2004e). Whey solids should not exceed 5–8% or sourness may result. PCF must not contain more than 44% moisture or less than 23% milk fat (FDA 2004e).

25.2.3 Pasteurized Process Cheese Spread

Pasteurized process cheese spreads (PCS) are similar to PC and PCF; however, the products contain more water, which makes them more spreadable. In addition to more allowable water, PCS are permitted added carbohydrates, including corn syrup solids, starches, sugars, and gums, not to exceed 0.8% by weight of the finished food (FDA 2004f). PCS must contain more than 44%, but less than 60% moisture and not less than 20% milk fat (FDA 2004f).

25.2.4 Pasteurized Process Cheese Product

Pasteurized process cheese product is a process cheese spread that may exceed the maximum moisture and/or does not meet the established minimum fat content (Dairy Management 1997).

25.2.5 Cold-Pack Cheese

The Code of Federal Regulations (FDA 2004b) defines cold-pack and club cheese as "the food prepared by comminuting, without the aid of heat, one or more cheeses of the same or two or more varieties, for manufacturing, into a homogeneous plastic mass" (FDA 2004b). The definition excludes cream cheese, Neufchatel cheese, cottage cheese, low-fat cottage cheese, cottage cheese dry curd, hard grating cheese, semisoft part-skim cheese, part-skim spiced cheese, and skim milk cheese. Further, all cheeses used in a cold-pack cheese must be made from pasteurized milk or must be held for not less than 60 days at a temperature of not less than 1.7°C before being comminuted (FDA 2004b).

Cold-pack cheeses (CPC) are composed of natural cheeses, ground finely, then mixed with vinegar or lactic, citric, acetic, or phosphoric acid, water, salt, color, and spices. The pH should not be below 4.5. Moisture must not exceed the maximum legal limit for the variety of natural cheese from which it is made, or the average of the maximum legal limits if more than one variety is used, but in no case more than 42% (FDA 2004b). However, the moisture content of a CPC made from two or more of the varieties Cheddar cheese, washed curd cheese, Colby cheese, and granular cheese is not more than 39% (FDA 2004b).

Fat in the solids must not be less than the legal minimum for the variety of natural cheese used, or the average for the legal minimum of cheeses used, but in no case less than 47% (FDA 2004b). However, the fat content of the solids of cold-pack Swiss cheese must not be less than 43% (45% if two or more varieties are used), and the fat content of the solids of cold-pack Gruyere cheese must not be less than 45%.

25.2.6 Cold-Pack Cheese Food

A cold-pack cheese food (CPF) is similar to a CPC, except a sweetening agent, such as sugar, dextrose, corn sugar, corn syrup, corn-syrup solids, glucose syrup, glucose-syrup solids, maltose, malt syrup, and hydrolyzed lactose, may be used in a quantity necessary for seasoning and to reduce water activity, which is necessary in the higher-moisture-content product. Other optional ingredients include cream, milk, skim milk, buttermilk, cheese whey, any of the foregoing from which part of the water has been removed, anhydrous milk fat, dehydrated cream, skim milk cheese for manufacturing, and albumin from cheese whey. The total weight of cheese or cheeses must constitute not

less than 51% of the weight of the finished CPF. Moisture must not exceed 44% and fat must be at least 23% in solids. If fruits, vegetables, or meats are added, the milk-fat content must be at least 22%. The pH must not be less than 4.5. Additionally, guar gum and/or xanthan gum may be used, but the total quantity must not exceed 0.3% of the weight of the finished food. When one or both such optional ingredients are used, dioctyl sodium sulfosuccinate may be used in a quantity not in excess of 0.5% by weight (FDA 2004c).

25.2.7 Cold-Pack Cheese Spread

Cold-pack cheese spreads (CPS) are not defined in the CFR, and do not have a standard of identity, but they may be found in the market. CPS may exceed the maximum moisture and/or not meet the established minimum fat content for CP and CPF. CPS are made by blending natural cheeses without the aid of heat and have a smooth spreadable body. CPS may contain herbs, spices, and other dairy ingredients, as well as gums for improving viscosity and mouthfeel, and acidulants to increase shelf-life and inhibit microbial growth.

25.3 PRINCIPLES OF PROCESSING

A schematic of the general process of pasteurized process cheese production is outlined in Figure 25.1. Ingredients and steps are detailed in the following sections.

25.3.1 Ingredients

25.3.1.1 Cheeses. Natural cheeses for pasteurized process and cold-pack cheeses are typically selected by trained personnel, based on age and quality, and combined in proportions to yield desirable flavor. When two varieties of cheese are combined, the weight of each variety of cheese is not less than 25% of the total weight of both, except that the weight of blue cheese, Nuworld cheese, Roquefort cheese, or Gorgonzola cheese is not less than 10% of the total weight of both, and the weight of Limburger cheese is not less than 5% of the total weight of both. When made from three or more varieties of cheese, the weight of each cheese is not less than 15% of the total weight of all, except that the weight of blue cheese, Nuworld cheese, Roquefort cheese, or Gorgonzola cheese is not less than 5% of the total weight of all, and the weight of Limburger cheese is not less than 3% of the total weight of all. In the case of "American cheese", these limits do not apply to the quantity of Cheddar cheese, washed curd cheese, Colby cheese, and granular cheese because such mixtures are considered as one variety of cheese (FDA 2004d). The process of cheese selection and processing will be described later in this chapter.

Enzyme-modified cheeses are natural cheeses to which proteases and/or lipases are blended to intensify cheese flavor about 10 to 30 times (Caric and Kaláb 1999). After incubating until the desired flavor is attained, enzyme-modified cheeses are packaged as a paste or dried to powder form. Enzyme-modified cheeses are especially flavorful and are used in a variety of food applications (Dairy Management 1997). Because of the long shelf-life of enzyme-modified cheeses (approximately 6 months), pasteurized process and cold-pack cheeses are a good application for them. Enzyme-modified cheeses can be used legally and help provide uniformity of cheese flavor across numerous lots of pasteurized process cheeses. Enzyme treatment also makes the cheese react better in the cooker and yields a product with superior body and texture over nonmodified

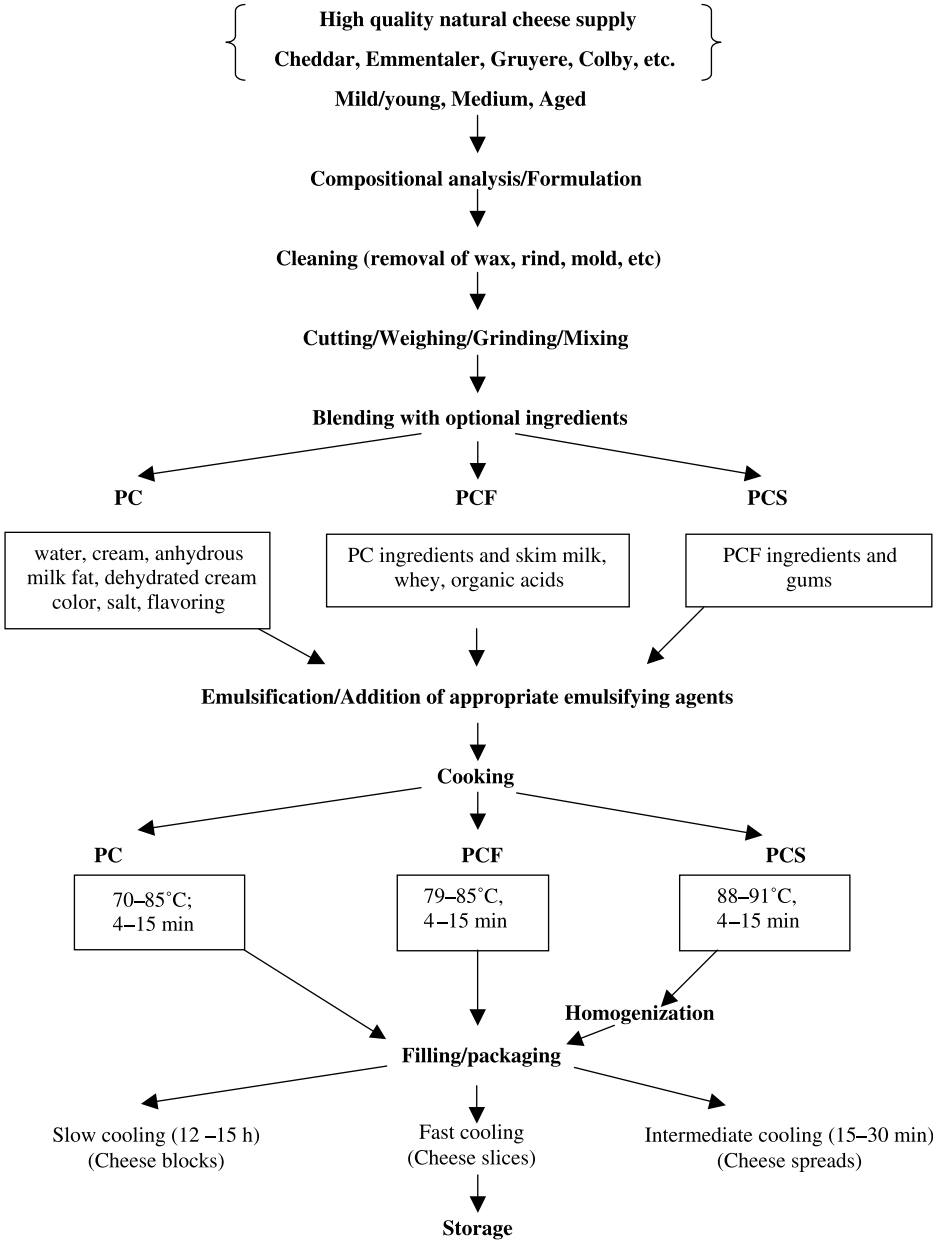

Figure 25.1 *General process steps for manufacture of pasteurized process cheese.*

cheese. After the enzymes have fulfilled their purpose (based upon target time, flavor, or chemical measure) the enzyme-modified cheese is pasteurized and the enzymes are inactivated.

25.3.1.2 Emulsifying Agents. The most common emulsifiers used in pasteurized process cheeses, foods, and spreads include various phosphate and citrate salts

TABLE 25.2 General Properties of Emulsifying Salts and Their Effect on Cheese Processing.

Property	Citrates	Ortho-phosphates	Pyro-phosphates	Polyphosphates (from 3 to 10 phosphate groups)	Aluminum Phosphates
Ion exchange (calcium sequesterization)	Low	Low	Moderate	High to very high	Low
Buffering action in pH range 5.3–6.0	High	High	Moderate	Low to very low	Nil
Para-casein dispersion	Low	Low	Very high	Very high to low	Very low
Bacteriostatic	Nil	Low	High	High to very high	Nil

Sources: Fox and others 1996; Meyer 1973.

(Table 25.2; Kosikowski and Mistry 1997). Emulsifying agents are not utilized in cold-pack cheese because no heating is involved in the process. The emulsifying agents that may be used include any one or any mixture of two or more of the following: monosodium phosphate, disodium phosphate, dipotassium phosphate, trisodium phosphate, sodium metaphosphate (sodium hexametaphosphate), sodium acid pyrophosphate, tetrasodium pyrophosphate, sodium aluminum phosphate, sodium citrate, potassium citrate, calcium citrate, sodium tartrate, and sodium potassium tartrate. The weight of the solids of emulsifying agent(s) must not exceed 3% (Kosikowski and Mistry 1997) of the weight of the PC.

Emulsifiers have many functions in pasteurized process cheeses, foods and spreads, including:

1. Removal of calcium from the protein system;
2. Peptizing, solubilizing, and dispersing proteins;
3. Hydrating and swelling proteins;
4. Emulsifying fat and stabilizing the emulsion;
5. Controlling and stabilizing pH;
6. Buffering capacity;
7. Bacteriostatic properties; and
8. Forming an appropriate structure after cooling

(Caric and others 1985; Kosikowski and Mistry 1997; Caric and Kaláb 1999). By dissolving protein, emulsifiers enable incorporation of fat, protein, and water into a uniform smooth mass (Kosikowski and Mistry 1997). Peptization also reduces the size of paracasein molecules, which creates the desired short texture of PC necessary for spreadability (Kosikowski and Mistry 1997).

Calcium-binding affinity is one of the most important functions of emulsifying agents because sequesterization of calcium improves flowability and smooth texture (Kosikowski and Mistry 1997; Caric and Kaláb 1999). Calcium-binding affinity increase in the following order:

$$\text{citrates} < \text{monophosphates (orthophosphates)} < \text{pyrophosphates} < \text{polyphosphates}$$

Differences in calcium affinity influence characteristics of the emulsification and melting properties of the final cheese (Metzger 2004). Additionally, colloidal calcium is replaced with sodium in casein micelles, which improves emulsifying characteristics and spreadability (Kosikowski and Mistry 1997). Condensed phosphates, including tetrapotassium diphosphate, disodium diphosphate, trisodium diphosphate, tetrasodium diphosphate, and so on, are particularly good at binding polyvalent cations (Berger and others 1989). They also peptize, disperse, and hydrate proteins, and promote and form stable emulsions (Berger and others 1989).

Although citrates lack bacteriostatic activity, monophosphates and polyphosphates, in particular, are quite effective against bacteria, including *Clostridium botulinum* (Caric and Kaláb 1999). Karahadian and others (1985) demonstrated that when disodium phosphate, trisodium citrate, dipotassium phosphate, tripotassium citrate, and sodium aluminum phosphate were used as single emulsifiers in PCF and PCS, they allowed toxin formation in many of the samples prepared. However, some inhibition of toxin formation was indicated for samples emulsified with disodium phosphate, and possibly trisodium citrate.

Typically, two or more emulsifiers are used in a formulation to combine desirable characteristics of each (Metzger 2004). In general, polyphosphates yield PC with better structure and keeping quality than other emulsifying agents because of their ability to solubilize calcium paracaseinate (Caric and Kaláb 1999). Pyrophosphates and orthophosphates contribute undesirable sensory attributes like tart flavor, very firm body, and calcium phosphate crystals to PC, so usage level should be minimized (Caric and Kaláb 1999). Although citrates are effective emulsifiers, they are ineffective as bacteriostatic agents (Caric and Kaláb 1999). In general, citrates are used for sliced process cheese products, whereas monophosphates, pyrophosphates, and polyphosphates are used for blocks, spreads, and sauces (Metzger 2004). Although citrates are appropriate for firm cheese with good melting properties, they are not suitable for spreadable PC because of limited creaming abilities and little tendency to absorb moisture (Meyers 1979). Monophosphates also provide good firming, buffering, and melting characteristics and are appropriate for sliced PC (Kosikowski and Mistry 1997). Short-chain polymeric phosphates (polyphosphates or condensed phosphates) provide good creaming, buffering and protein solubility attributes (Kosikowski and Mistry 1997). Long-chain polyphosphates provide firm body, but the resulting product does not melt easily because of excellent ion exchanging and protein solubility properties while having limited creaming properties (Meyers 1997).

The amount of emulsifying agents to use in a formulation varies with intact casein content; less emulsifying agent is necessary when less intact casein (aged cheese) is available (Berger and others 1989). Adding too much emulsifying agent may result in a viscous cheese with firm consistency, crystallization of emulsifying agent, oiling off, and emulsifier flavor. Using too little emulsifying agent can yield a thick, poorly flowing, nonhomogeneous mass that is difficult to pump.

25.3.1.3 Optional Ingredients. Pasteurized process cheeses, foods, and spreads may contain one or more of the allowed optional ingredients, including water, cream, anhydrous milk fat, dehydrated cream (in any combination or quantity as long as the weight of the fat derived from them is less than 5% of the weight of the PC), salt, harmless artificial coloring, spices, or flavorings (other than any that singly, or in combination with other ingredients, simulate the flavor of a cheese of any age or variety). In the

case of cold-pack cheeses, cream, anhydrous milk fat, and dehydrated cream are disallowed. Pasteurized process and cold-pack cheeses may contain an acidifying agent consisting of one or any mixture of the following: vinegar, lactic acid, citric acid, acetic acid, and phosphoric acid, as long as the pH of the PC is not below 5.3 and the pH of the finished cold-pack cheese is not below 4.5.

Although listed as an optional ingredient, water will inevitably be incorporated during the production of PC, PCF, and PCS because it is needed to hydrate and dissolve emulsifying agents (Meyer 1973). Water must be clear, free of heavy-metal contaminants, odorless, flavorless, and either sterile or nearly free of microorganisms (Meyer 1973).

Although not allowed in PC, optional ingredients allowed in PCF and PCS include various forms of non-fat milk and cheese whey. Skim milk powder, when not used in excess of 12% of the total mass, can improve the spreadability and stability of PC (Caric and Kaláb 1999). To avoid browning, total lactose content of the PC formulation should not exceed 4% (Kapoor and Metzger 2004) to 6% (Caric and Kaláb 1999). Another drawback of lactose is its tendency to form crystals when the concentration of lactose in the water phase of PC exceeds 14% (Metzger 2004), because the maximum solubility of lactose in water is 17% (Harper 1992). Whey products with reduced mineral and/or reduced lactose content are preferable because they yield PC with better flavors and color (Caric and Kaláb 1999). However, because whey proteins denature and form gels at high temperatures, they have the potential to reduce the melting quality of PC (Metzger 2004). Melting quality deteriorates when whey protein is incorporated at a rate exceeding 2% (as actual whey protein) in a formulation incorporating 45% moisture (Metzger 2004).

Gupta and Reuter (1992) demonstrated that the addition of approximately 2.2% whey protein concentrate (20% whey proteins and 5.8% lactose) in the final PCF (45% moisture) did not affect the quality of the product. Kapoor and Metzger (2004) manufactured PC, PCF, and PCS, with a control formula and a salt whey formula produced for each. The salt and water in the cheese formula were replaced with filtered and pasteurized salt whey, an underutilized natural cheese byproduct, in the salt whey formula. There were no significant differences in composition or functionality between the cheese formula and salt whey formula within each variety of processed cheese, indicating that salt whey can be used as an ingredient in PC without adversely affecting process cheese quality.

For PCS, quantities of 0.1 to 0.25% hydrocolloids (guar gum, gum arabic, gelatin, carrageen, alginate, agar and pectin) yield desirable water-binding properties and impart the smooth mouthfeel expected in these products (Berger and others 1989).

PC slices or cuts in consumer-sized packages may contain lecithin as an optional antisticking agent in an amount not to exceed 0.03% by weight of the finished product. Additionally, mold-inhibiting ingredient(s) may be used. PC may consist of not more than 0.2% (0.3% for cold-pack cheeses) by weight of sorbic acid, potassium sorbate, sodium sorbate, or any combination of two or more of these, or not more than 0.3% by weight of sodium propionate, calcium propionate, or a combination of sodium propionate and calcium propionate (FDA 2004d).

Nisin is an antimicrobial agent that is effective against most Gram-positive microorganisms, including *Clostridium* and *Bacillus* species (Meyer 1973; Berger and others 1989; Infanger 2004). Nisin is generally regarded as safe (GRAS) for process cheese applications in the United States at usage levels between 200 and 600 mg/kg (Infanger 2004). Production of Cheddar cheese with nisin-producing lactococci instead of

nisin itself, avoids the necessity of special package labeling. Zottola and others (1994) made Cheddar with nisin-producing lactococci then produced PCS and CPS with the cheese. The spreads were inoculated with pathogens and evaluated over time. Authors concluded that use of nisin-containing cheese as an ingredient in PCS or CPS could be an effective method of controlling the growth of undesirable microorganisms in these processed foods.

25.3.2 Production

25.3.2.1 Selection and Analysis. A large stock of natural cheeses, ranging from mild to sharp in flavor, is necessary for the production of pasteurized process cheeses. Experienced selectors are needed to identify flavor and body characteristics of natural cheeses to be selected for blend uniformity. Additionally, natural cheeses with microbial defects should not be selected for PC. Spore-forming, gas-producing, and pathogenic bacteria are particularly undesirable (Caric and Kaláb 1999).

Various authors recommend a variety of formulations for PC. Thomas (1977) recommended 70–75% mild natural cheese be combined with 25–30% medium to mature natural cheese. Caric and Kaláb (1999) recommended 30–40% young cheese, 50–60% mild to medium cheese, and 10% mature cheese for an elastic PC that is ideal for slices. Kosikowski and Mistry (1997) encouraged the combination of 55% young, 35% medium-aged, and 10% aged cheese for optimum firmness and slicing qualities. Essentially, the formulation is determined based on the quality and consistency desired in the final product.

One of the main factors dictating body/texture consistency is relative intact casein content. The level of intact casein influences the structure of the final cheese product, and must be considered when selecting cheeses for PC, PCF, and PCS. Only the intact casein participates in the formation of the emulsified casein network; thus, natural cheeses are selected to achieve a target amount of intact casein in the process cheese. Intact casein is highest in young cheese. PC products made with high intact protein have a long filament-like structure, which yields a product that is sliceable or rubbery, depending on the moisture content (Meyer 1973). A low intact protein content (high content of hydrolyzed peptides and amino acids), resulting from extensive proteolysis in aged cheeses, produces a short structure, appropriate for spreads (Meyer 1973). Rennet-set cheeses of only a few days old possess 90–95% intact casein. Intact casein in mold-ripened cheeses may decline to 30% within a couple of months of aging (Berger and others 1989). In Emmental, intact casein declines from about 88% after one month, to 75–80% after six months, and 70–75% after nine months of aging (Berger and others 1989). The content of intact casein in the finished product should not fall below 12% (Berger and others 1989). For block PC intended for slicing and elasticity, the natural cheeses must have predominantly high intact casein ($\geq$70%) (Meyer 1973). Intact casein can also be obtained from other dairy sources, including non-fat dry milk or milk protein concentrate (Metzger 2004). A summary of recommended combinations of raw materials for different PC products is included in Table 25.3.

A high content of young cheese in the blend will enable formation of a stable emulsion with high water-binding capacity, firm body, and good slicing properties (Thomas 1977; Caric and Kaláb 1999). However, PC with a high content of young, mild natural cheese will have less flavor than PC formulations that include aged cheese, and will exhibit a tendency to harden during storage (Caric and Kaláb 1999). A high content of aged cheese in

TABLE 25.3 Recommended Combinations of Raw Materials for Different Types of Pasteurized Process Cheeses.

Type	Young Cheese (%)	Medium-Ripe Cheese (%)	Aged Cheese (%)
Pasteurized process cheese for slicing, firm	50–60	30–40	10
Pasteurized process cheese slices	60	30	10
Pasteurized process cheese for spreading	30	50	20
Pasteurized process cheese spread	60	40	0

Source: Meyer 1973.

the PC blend will result in more highly flavored PC, good flow properties and high melting index (Thomas 1977; Caric and Kaláb 1999). However, the final product may have a sharp flavor, low emulsion stability, and soft consistency (Caric and Kaláb 1999).

Cheese stocks are analyzed for fat and moisture, then those values are used to calculate emulsifying agents, condiments (flavors), salt, water, and other ingredients to be used, so that the finished cheese will meet government regulations (Kosikowski and Mistry 1997). As there are so many formulation parameters that are important for process cheese functionality and cost, the task of creating a process cheese formulation is made much easier with the aid of a computer program that can track the important formulation parameters as well as the cost of a formula under consideration. Most process cheese manufacturers use a formulation program to assist in the formulation process and cost control (Metzger 2004).

CP cheeses are commonly served as a table spread or used by foodservice as an ingredient, because no heat treatment is given to the product during processing. According to manufacturers of process and cold-pack products, CP and CPF have fresher and more natural flavor when compared to PC (Anon. February 1999). Although selection and preparation of cheeses for use in CP and CPF are quite similar to selection for PC and related products, the major disadvantage is that cold-pack products do not have the same smooth consistency as PC. The body and consistency of CP and CPF depend on the selection of cheese ingredients and the process. Aged cheese will provide a smoother texture compared to young cheese (Anon. February 1999). Similarly, the amount of shear during CP and CPF processing will determine the particle size – the higher the shear, the smoother the product (Anon. February 1999). The pH values for CP and CPF are typically between 5.0 and 5.2 (Duitschaever and Irvine 1973). At pH values below 5.0, syneresis may occur because of proximity to the casein isoelectric point, the point of minimum hydration (Duitschaever and Irvine 1973).

25.3.2.2 Preparation. After selection for pasteurized or cold-pack applications, cheese surfaces are trimmed by scraping and cleaning (wax, rinds, mold spots discarded) (Kosikowski and Mistry 1997). A separate room from the processing floor should be used for cleaning to minimize contamination of products (Berger and others 1989). After proper cleaning, natural cheeses are pre-cut to a manageable size. Portions are shredded or otherwise reduced to about 10–20 mm in diameter, by grinding or grating, because fine particles go into solution more efficiently and completely than large ones (Meyer 1973;

Kosikowski and Mistry 1997). In the largest plants, minced cheese is weighed automatically and conveyed to the mixing vessels (Berger and others 1989).

25.3.2.3 Mixing. Prior to cooking, minced cheeses should be mixed in a vertical or horizontal mixing vessel until a homogeneous mixture is obtained (Berger and others 1989). Emulsifying agents can be added during mixing, prior to or during the cooking stage (Berger and others 1989). The pH of a PC should typically be between 5.6 and 5.8, between 5.4 and 5.6 for a PCF, and about 5.2 for a PCS (Caric and Kaláb 1999). The pH can either be adjusted with the application of acid, base, or emulsifying agent(s). At or below pH 5.0, PC becomes crumbly, while above pH 6.5, PC becomes excessively soft and microbiological problems may result (Caric and Kaláb 1999). Adjustment of pH values between 5.7 and 6.0 during manufacture is recommended for optimum elasticity and springiness of the final product (Marchesseau and others 1997). Trisodium citrate, monosodium phosphate, and disodium pyrophosphate are effective at lowering pH, and disodium phosphate, tetrasodium pyrophosphate, and polyphosphates effectively raise pH (Caric and Kaláb 1999). After blending, material is then taken to the cooker by conveyer belts, worm-feed systems, or other means.

25.3.2.4 Cooking. During cooking, cheeses and other ingredients are heated to high temperatures, in either steam-jacketed kettles or direct steam injector stainless-steel cookers to attain uniformity, safety, and to extend keeping quality. In batch processes, the blend is heated to between 70 and 95°C for 4–15 min (Meyer 1973; Kosikowski and Mistry 1997; Caric and Kaláb 1999). Temperatures of 70–75°C are needed for melting, and creaming is optimal between 80 and 90°C (Berger and others 1989). Sometimes temperatures up to 145°C are used as anaerobic bacterial spores are destroyed above 140°C (Berger and others 1989). Shorter times can be utilized for processing block PC, but longer times ensure spreadable consistency, which is needed for PCF and PCS (Berger and others 1989). Legally, PC should be heated for not less than 30 s at a temperature of not less than 65.6°C (FDA 2004d). When tested for phosphatase, the phenol equivalent of 0.25 g of PC must not be more than 3 μg (FDA 2004d).

Conventional batch-processing cookers are composed of a cast-iron supporting frame or a supporting column, two stainless-steel melting pans with matching lids fitted for steam, vacuum, or water injection, a stirrer, and a tipping mechanism (Berger and others 1989). Raw materials are added to the pan, followed by optional ingredients, then emulsifying agents (if not added at mixing) and water (Berger and others 1989). In continuous-processing cookers, equipment consists mainly of horizontal and/or vertical stainless-steel pipes inside which the cheese mass is continuously fed through single- or double-worm feeders from the input funnel to the outlet and is melted by exposure to direct steam at temperatures from 130 to 145°C for 2–3 s (Meyer 1973; Berger and others 1989; Kosikowski and Mistry 1997). In any case, time, temperature, and stirring rate must be monitored to minimize defects and ensure uniformity.

During emulsification and cooking, the critical steps involve conversion of the calcium cross-linked casein network that is present in natural cheese into a hydrated gel (Fig. 25.2). Initially, organic phosphate groups, which are covalently attached to casein, interact with calcium and inorganic phosphate, which allows several casein molecules to bind together (Metzger 2004). Because of these protein–protein interactions, and lack of interaction between fat, water, and proteins, if natural cheese is heated, it will release a large amount of free fat and water (Metzger 2004). The melted cheese in the cooker initially

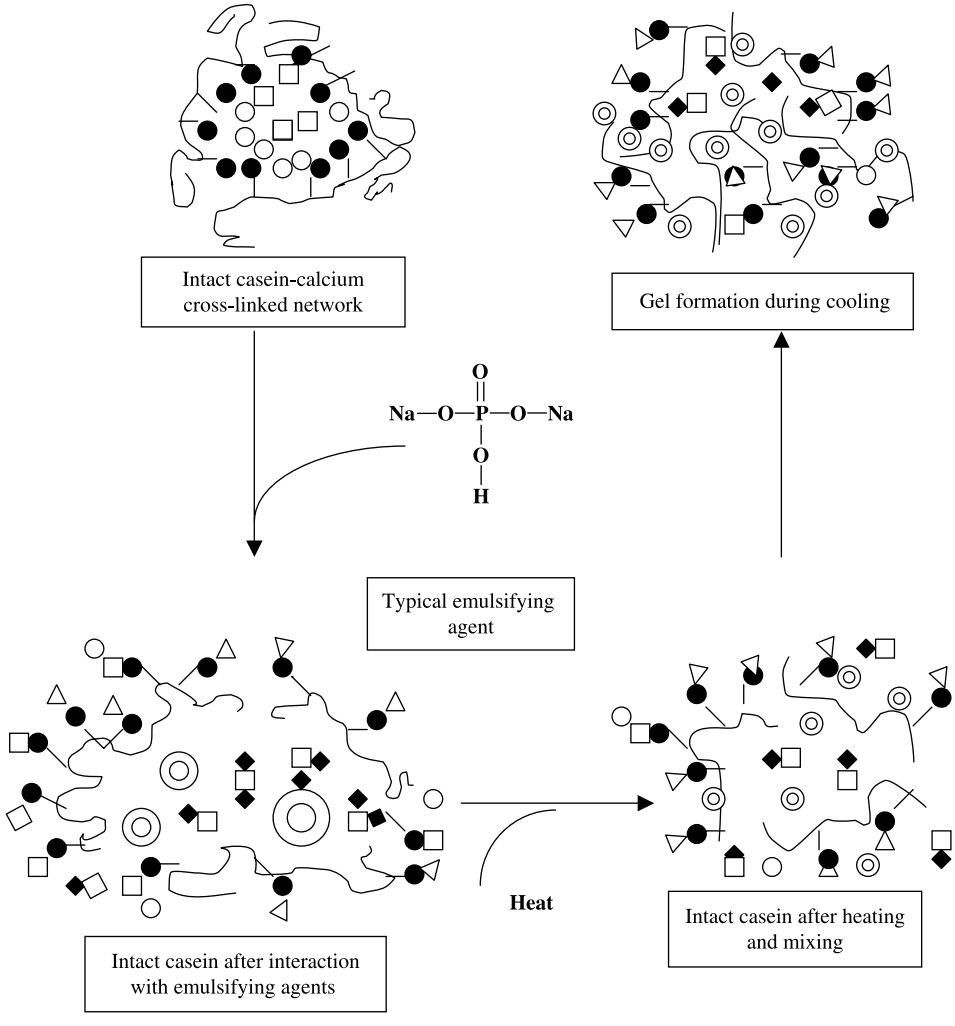

Figure 25.2 *Schematic of pasteurized process cheese emulsification; symbols represent: calcium (□), inorganic phosphate (○), organic phosphate (●), sodium ions (△), emulsifying agents (◆), and milkfat (◎) (Metzger 2004).*

separates into a fat and serum phase until the introduction of suitable emulsifying agents. The resulting upward pH shift solubilizes the protein, resurfaces the fat, and gives a smooth homogeneous mass. Some emulsifiers also peptize the protein molecule under the conditions of processing, to give a desirable short-grain texture.

When emulsifying agents are added to natural cheese, they are hydrated and release sodium ions (Fig. 25.2). The emulsifying agents contain negatively charged groups, which have the potential to interact with positively charged calcium ions, essentially pulling the calcium from the caseins (Metzger 2004). Because the casein then has free negative charges, sodium ions that were released by the emulsifying agents replace the calcium, resulting in a structural change in the previously calcium-cross-linked casein network (Fig. 25.2). The interchange between calcium and sodium ions is a critical

step. Although sodium ions act dispersively, and unwind, dissolve, and peptize proteins, calcium ions dehydrate, condense, and build larger aggregates through polymerization of polypeptides (Meyer 1973). The structural exchange results in elimination of some of the protein–protein interactions; the hydrophobic portions of caseins may interact with fat and the hydrophilic portions can interact with water (Metzger 2004).

Heat and mixing induce interactions between emulsified casein and fat and water, which results in a homogeneous melted mass (Fig. 25.2). During cooling, protein–protein interactions among emulsified casein molecules yield a cross-linked gel (Metzger 2004).

Great skill is involved in knowing when the cooking process is complete. Production of a PC with an appealing short structure with good spreading properties, from a combination of mature and young cheeses with a high level of intact protein, is referred to as "creaming" (Berger and others 1989). Production of spreadable cheese starts with a combination of natural cheeses with 60–80% intact casein (not below 50%), because short texture is desired. If a block cheese with good slicing properties is desired, proper creaming and a high proportion of young cheese with 70–90% intact casein is required. "Overcreaming" is a term describing product consistency ranging from pudding-like to solid.

Higher heat and lower pH are utilized in the production of PCF to prevent bacterial spoilage, which is accelerated at higher water activities (Kosikowski and Mistry 1997). For spreadability, PCS production requires even higher processing temperature and lower pH than PCF because of the even higher water content than PC (Kosikowski and Mistry 1997).

Other components, such as meat products, spices, fruits, or vegetables, may be added at the beginning or towards the end of the cooking process of PCS (Meyer 1973). An advantage to adding inclusions at the beginning is bacteriological safety, although adding at the end prevents shattering of susceptible fragile components (Meyer 1973). Spices are typically added at the beginning of cooking, to ensure even distribution and pasteurization (Berger and others 1989).

Cold-pack cheeses undergo no cooking step, so will not be discussed in this section. Thus, all ingredients must be pasteurized or aged $\geq 1.7^{\circ}C$ for ≥ 60 days prior to grinding, mixing, and packaging (FDA 2004b).

25.3.2.5 Homogenization. Homogenization may be employed to ensure smoothness in the final product, but it is typically reserved only for high-fat PC, PCF, or PCS products (Meyer 1973). When utilized, homogenization is carried out immediately after cooking has been completed, and certain fragile additives, such as meats and vegetables, must be added after this step (Meyer 1973). Thus, intense care should be taken to prevent post-pasteurization contamination.

25.3.2.6 Packaging and Cooling. Heating, emulsification, and unloading of the molten cheese require only a few minutes. If the process is delayed, oiling off, body thickening, browning, and other undesirable defects may result. At the proper time, the blended smooth-textured hot mass can be ejected from the cooker into hoppers or by means of a pump and pipelines, then packaged by high-speed machines that are typically housed in a separate room (Meyer 1973; Kosikowski and Mistry 1997). When the cheese becomes velvety smooth the cheese mass is ready for withdrawal at pasteurization temperature ($71^{\circ}C$) (Berger and others 1989). The smooth, molten cheese must be unloaded from the cookers and pumped into packaging hoppers quickly yet gently to prevent either a temperature drop or overcreaming (Berger and others 1989; Kosikowski and Mistry 1997).

In most facilities, filling and packaging of emulsified liquid mass is automated (Berger and others 1989). Most packaging is consumer portioned from 30 to 225 g, and block cheese is filled into 200 g to 2.5 kg portions (Berger and others 1989; Kosikowski and Mistry 1997). Cheeses are sealed in properly lacquered aluminum foil and inserted into outer packaging at up to 800 units per min (Berger and others 1989). Filled and sealed PC containers are placed in packing cases, which are then turned upside-down (to prevent bubbles) and held at room temperature for 4–6 h before cold storage.

Manufacturers have several options for producing PC in slices, including the block, roller, strip, or injection methods (Berger and others 1989). In the block method, cooled blocks are cut and packed into molded plastic pouches with or without separating foil between each slice (Berger and others 1989). In the roller method, the hot melted cheese emulsion is distributed uniformly on chilled steel rollers, where a wide thin mat of cheese, or thin bands of cold solidified cheese are formed (Meyer 1973; Kosikowski and Mistry 1997). The continuously produced strips are cut by rotary knives to consumer size, laid above each other in layers of eight or more, divided, cut, and automatically wrapped (lecithin is permitted to reduce sticking) (Meyer 1973; Kosikowski and Mistry 1997). The strip or band method is similar to the roller method, except that it employs a moving belt made of stainless steel instead of a cooling roller (Berger and others 1989). The injection method involves injection of the cheese into preshaped body foil, which is pressed flat into the shape of a slice, then cut and packaged into multipacks (Berger and others 1989). Vacuum packaging, or evacuating the air and replacing with an inert gas, prevents spoilage (Meyer 1973).

The USDA requires that PC be cooled to below 38°C within 24 h of manufacture (Zehren and Nusbaum 1992). Cooling is conducted relatively rapidly by storing portion-filled boxes on open shelves, where they can be exposed to cold air, or by immediately conveying boxes through a cooling tunnel on a belt (Berger and others 1989). The storeroom for finished products is generally maintained at temperatures between 12 and 15°C, which is cool enough to prevent further creaming (Berger and others 1989).

Cold-pack cheeses are packaged in various containers, including sausage-shaped casings, plastic or waxed cardboard cups, metal foil, and so on, which are designed to keep air out (USDA 1978).

25.4 PRODUCT ATTRIBUTES

25.4.1 Nutrition

In principle, a PC, PCF, and PCS will have the same nutritional composition as the cheese from which it was made (Berger and others 1989). Pasteurized process cheese and related products contribute high-quality protein, fat, fat-soluble and water-soluble vitamins, calcium, phosphorus, and other nutrients to the diet (Berger and others 1989). Ha and others (1989) demonstrated that conjugated linoleic acid (CLA) content of Cheddar cheese and PC were similar and approximately double the total CLA content of milk. Additionally, total CLA content of a PCS enriched with whey concentrate (Cheese Whiz™) was four to five times higher than that of PC (Ha and others 1989). CLA has been shown to exhibit anticarcinogenic activity (Ha and others 1989; Parodi 1999; Park and others 2004).

Considering the high consumption of PC, PCF, and PCS by Americans, these products may serve as good vehicles for delivering nutritive components to consumers. For instance, dairy products contribute little iron to human diets (Zhang and Mahoney 1991). By showing that consumers gave similar hedonic scores to iron-fortified (Fe-casein, Fe-whey protein, or $FECl_3$) and control cheeses Zhang and Mahoney (1991) demonstrated that it is possible to produce good-quality, iron-fortified pasteurized process Cheddar cheese.

25.4.2 Quality

A good PC has a smooth, compact body, devoid of fermentive gas holes, and a uniform color (Kosikowski and Mistry 1997). PCF is similar to PC in quality, but it has a softer body and milder flavor because of the higher water content (Kosikowski and Mistry 1997). PCS has even more water than PCF and PC, so it is even softer and more spreadable. If air cells are present, they are typically very small and are concentrated on the bottom of the package (Kosikowski and Mistry 1997). High-quality PC should have a clean, pleasant flavor and can be sliced without crumbling or sticking, and it should melt uniformly and smoothly (Kosikowski and Mistry 1997).

Of course, for quality PC, PCF, and PCS, all ingredients used in manufacture must be of high quality. Poor-quality milk fat can yield products with oxidized or rancid flavors, and poor-quality milk protein can yield PC with a bitter taste. However, use of quality ingredients does not guarantee a high-quality final product; appropriate formulation and proper manufacturing conditions are critical to quality pasteurized process cheeses, foods, and spreads. Some of the defect attributes that may be observed in pasteurized process cheese products are summarized in Table 25.4.

The major processing conditions that influence PC products are the mixing (shear) conditions, cook temperature, and cooling rate (Metzger 2004). The mixing conditions used during processing influence the interaction between the emulsified casein and fat. In general, as mixing rate and time are increased, the interactions between the emulsified casein and fat also increase. In a similar fashion, as processing temperature increases, the interactions among the hydrophobic portions of the emulsified casein and fat increase. Increasing the mixing rate or increasing the cooking temperature will typically increase the firmness but decrease the meltability of PC. During cooling, the emulsified caseins interact to form a gel, and the rate of cooling will influence the characteristics of the gel that is formed. A rapid cooling rate causes formation of a porous gel with weak cross-links, whereas a slow cooling rate produces a less porous gel with strong cross-links. A less porous gel with strong cross-links will produce a firm PC with less meltability. Consequently, a slow cooling rate will produce a firm PC with less meltability whereas a fast cooling rate will produce a softer PC with more meltability. The importance of processing conditions and cooling rate are so critical that is it is possible to take the same formulation and produce several process cheeses with completely different functionality by simply adjusting the manufacturing conditions (Metzger 2004).

25.4.3 Shelf-Life and Safety

The shelf-life of pasteurized process cheese and related products varies between 8 weeks (slices) and 20 weeks (small portions) to 1 year (tubes or cans) at room temperature (Schär and Bosset 2002). During storage, even bacteriologically stable products in good

TABLE 25.4 Common Attributes in Pasteurized Process Cheeses, Identification, and Their Probable Causes.

Attribute	Probable Cause	Prevention
Acid or bitter taste	Excess phosphates	• Minimize phosphate usage level • Natural cheese source(s) • Carefully select cheeses for base formula
Browning	Salmon or tan discoloration	• Avoid cooking above 85°C in the presence of lactose (skim or whey powder)
Crumbly	Cheese falls apart upon slicing	• Optimize moisture and emulsifier(s) levels
Crystals and/or grainy or sandy texture	Protein precipitation, or excess polyphosphates, or tyrosine crystals, or crystals of calcium diphosphate lactose or insoluble emulsifier	• Control pH to 5.2, not lower than 5.0 • Minimize phosphate usage level • Avoid use of natural cheese with crystals • Cool packaged cheeses slowly • Avoid excessive use of skim or whey powder
Firm body	Cheese does not slice smoothly due to excess polyphosphates	• Minimize polyphosphateusage level
Gassy	Physical Air bubbles introduced during processing trapped in the body of processed cheese while cooking Microbial gas formation during storage due to presence of *E. coli* or bacterial spores	• Appropriate heat treatment of pasteurized process cheese • Proper selection of cheeses(s) with low spore counts • Proper formulation (moisture levels, pH, type and amount of emulsifier(s) and NaCl, and prior treatment of condiments • Use of approved antimicrobials and/or sorbate-coated foil
Heavy body	Gummy, dense cheese body due to excess cooking	• Optimize cooking time
Leaky	Fat and water separate due to improper emulsifier and pH	• Optimize emulsifier usage and pH
Moldy flavor	Mold growth; air contamination	• Properly vacuum-seal packages
Oxidized, rancid or unclean flavor	Off-flavors resulting from natural cheese	• Avoid use of natural cheese with off-flavors
Soft body	Oily, spongy body due to excess fat, citrates, or pH	• Optimize formulation with natural cheeses and emulsifying agents
Sticky	Cheese holds to knife blade	• Control moisture to below legal limits • Reduce pH to around 5.2

Source: Meyer 1973.

packaging undergo chemical and physical aging processes that impair flavor and texture of products (Schär and Bosset 2002). A comprehensive review by Schär and Bosset (2002) details processes that occur during storage, including loss of water vapor, hydrolysis of polyphosphates, changes in ionic equilibria, formation of crystals, reactions induced by

heat-stable enzymes, nonenzymatic browning, reactions induced by light and oxygen, and interactions with packaging materials.

Thapa and Gupta (1992) reported some of the changes in sensorial and rheological properties that occur in PC and PCF during storage. Body and texture acceptability scores of PCF and PC declined slightly during storage, as the cheeses became harder and drier. Appearance acceptability scores declined as a result of increased browning during storage. Authors attributed faster flavor deterioration in PCF compared to PC to the higher moisture content of PCF, because high moisture and water activity facilitate food spoilage. Finally, the shelf-life of PCF was shortened by replacing 20% of cheese solids with whey protein concentrate.

Research has suggested promise for reduced-fat PC, PCF, and PCS. Muir and others (1997) characterized the sensory quality of 16 commercial PCS samples and showed that texture and mouthfeel were influenced by fat content. On the other hand, no evidence of differences in flavor or spreadability were associated with fat content, suggesting that formulation manipulation may be more effective at homogenizing flavor and spreadablilty than texture and mouthfeel of PCS. Moreover, Brummel and Lee (1990) were able to improve the texture of 40 and 50% reduced-fat PCS by using soluble hydrocolloids. Spreads with 15% fat and with added hydrocolloids, lambda-carrageenan (2.2%), pectin (1.7–2.2%), or low-viscosity guar (1.7%) had textures consistent with the 25% fat control. Above these gum levels, cheese-spread firmness (by Instron measure) increased while melt decreased. A 15% fat, 62% moisture cheese spread with 1.7% pectin was most like the control having 25% fat. Swenson and others (2000) characterized the effects of emulsifying agents and hydrocolloids, cook time, cook temperature, and pH on firmness, meltability, and spreadability of fat-free PCS. Incorporation of hydrocolloids resulted in increased firmness and decreased melt. Increases in cook time generally produced softer, more meltable cheeses, and increases in cook temperature decreased firmness and increased meltability and spreadability. These findings imply that reduction of fat in PC products may require manipulation of processing conditions to maximize quality.

Glass and others (1998) evaluated commercial PC slices for the ability to support the growth of four foodborne pathogens, inoculated at 10^3 cfu/g, during 4 days of storage at 30°C. *Staphylococcus aureus* levels remained relatively constant during the testing period, and were below levels that support detectable enterotoxin production. Populations of *Listeria monocytogenes*, *Escherichia coli* O157 : H7, and *Salmonella* serotypes decreased during storage. Although these results imply inherent safety of PC, PCF, and PCS, of course, post-pasteurization contamination of any kind should be minimized by using good manufacturing and handling practices to ensure safety of PC, PCF, and PCS products.

As CPC, CPF, and CPS are not heat treated or aged after manufacture, contamination of starting materials with pathogens is of major concern. Park and others (1970) contaminated CPF with *Salmonella typhimurium* and found that the pathogen survived up to 19 weeks, in the presence of preservative, if pH was not adjusted to 5.0 with acid. Ryser and Marth (1988) investigated the ability of *L. monocytogenes* to persist in various formulations of CPF manufactured with or without preservatives and/or acidifying agents. *L. monocytogenes* was unable to grow in CPF at pH 5.0–5.45, regardless of the formulation. It is not surprising that *L. monocytogenes* survived the longest in nonacidified CPF made without preservatives. *L. monocytogenes* numbers declined only 0.6–1.55 log at 4°C during the six-month shelf-life of the product. Because salt lowers the water

activity of a food, it indirectly enhances the effectiveness of preservatives; thus, the authors concluded that combining salt and preservatives in CPF at pH 5.0 can enhance destruction of *L. monocytogenes.* Of course, manufacturing CPC, CPF, and CPS with pathogen-free ingredients, under the best sanitary conditions, is of utmost importance for food safety. However, incorporation of preservatives, especially sorbic acid, and small amounts of lactic and/or acetic acid to decrease the pH to 5.0, decreases the chance for survival of *L. monocytogenes* and can provide additional assurance of safety of CPC, CPF, and CPS (Ryser and Marth 1988; Park and others 2004).

25.5 SUMMARY/CONCLUSIONS

The extended shelf-life of pasteurized process cheeses compared to natural cheeses, combined with the equivalent nutrition and enhanced convenience and consistency of these products, make pasteurized process cheese and related products popular foods in the human diet. Due to the variety of optional ingredients and advanced manufacturing practices available in the industry, plentiful opportunities exist for food scientists who wish to improve the diversity and nutritional quality of pasteurized process cheese and related products for eager consumers. With technical expertise, good sanitation practices, and creativity, great opportunities exist for a multitude of new high-quality, safe, and versatile pasteurized process cheeses and related products.

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26

Cottage Cheese

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26.1 Introduction 618
26.2 Production of Cottage Cheese 618
26.2.1 The Ingredients 618
26.2.1.1 Milk 618
26.2.1.2 Calcium 619
26.2.1.3 Cultures 619
26.2.1.4 Enzymes 620
26.2.1.5 Cream Dressing 620
26.2.1.6 Water 621
26.2.2 The Process of Cottage Cheese Making 621
26.2.2.1 Sanitation 621
26.2.2.2 Separation 622
26.2.2.3 Pasteurization 622
26.2.2.4 Homogenization 622
26.2.2.5 Culture Preparation 622
26.2.2.6 Culture Addition 623
26.2.2.7 Enzyme Addition 623
26.2.2.8 Cutting 623
26.2.2.9 Healing 624
26.2.2.10 Cooking 624
26.2.2.11 Washing 625
26.2.2.12 Creaming 626
26.2.2.13 Packaging and Shelf-Life 627

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

26.3 Evaluation of Cottage Cheese 628
26.4 Summary 632
References 633

26.1 INTRODUCTION

Cottage cheese is an unripened, soft, white cooked curd cheese made from skim milk (Code of Federal Regulations 2004). Cottage cheese may be found commercially in various forms, including dry curds, creamed cottage cheese, large curd, small curd, California-style, low-fat, nonfat, no salt, or blended with fruits, chives, or other inclusions. Except in the case of dry curds, a cream dressing is added to the nonfat cottage cheese curds, to achieve the appropriate fat content for the given product identity. Both cottage cheese and creamed cottage cheese must not contain more than 80% moisture (Watrous 1997; USDA 2001). Creamed cottage cheese must contain not less than 4% milk fat, unless labeled as 2%, 1%, or nonfat cottage cheese (Watrous 1997; USDA 2001). High-quality creamed cottage cheese containing about 1% salt should have a mildly acidic, sweet, clean flavor (Bodyfelt and others 1988; Potter 2004).

Cottage cheese has been made commercially in the United States since about 1915 (Kosikowsky and Mistry 1997a) and consumption peaked (at 2.5 kg per capita) in the 1970s (IDFA 2002). Since then, sales of creamed and low-fat cottage cheese have declined from 452 million kg (2 kg per capita) in 1980, to 333 million kg in 2000 (IDFA 2002). U.S. production of cottage cheese was over 167 million kg in 2000, but since 1996, per capita sales (1.2 kg) of cottage cheese have remained nearly unchanged (IDFA 2002). Many theories have been made on why cottage cheese sales have not increased. One theory is that the success of yogurt, especially with children, has overshadowed cottage cheese. Focus by dairy companies has been on promoting fluid milk and cultured products, with little emphasis being placed on cottage cheese. Another theory is that cottage cheese has long been associated with being a diet food. That would lend itself to a smaller targeted audience and then only if they are consistently dieting. A final theory is that the uniformity of cottage cheese is inconsistent, turning away consumers. In any case, to be successful in promoting and marketing cottage cheese, expectations of product quality are the basis for the product.

26.2 PRODUCTION OF COTTAGE CHEESE

26.2.1 The Ingredients

26.2.1.1 Milk. High-quality Grade "A" skim milk must be used to make cottage cheese curds because

1. It is the law,
2. High-quality cheese cannot be made from inferior milk, and
3. Fat softens the curd (USDA 2001; Code of Federal Regulations 2004)

The United States Department of Agriculture (USDA 2001) states that "Cottage cheese shall be manufactured and packaged in accordance with the applicable requirements

contained in 7 CFR Part 58, Grading and Inspection, General Specifications for Approved Plants and Standards for Grades of Dairy Products and inspected by the AMS, Dairy Grading Branch. Alternatively, cottage cheese may be manufactured and packaged in accordance with the applicable requirements contained in the latest revision of the 'Grade A Pasteurized Milk Ordinance', Food and Drug Administration" and "Cottage cheese and dry curd cottage cheese shall comply with all applicable Federal regulations including those contained in the Food and Drug Administration's Standard of Identity for Cottage Cheese 21 CFR Part 133.128 for cottage cheese or 21 CFR Part 133.129 for dry curd cottage cheese" (U.S. Department of Health and Human Services 1999; Code of Federal Regulations 2004).

The incoming raw milk must be of high quality with regard to aroma, flavor, titratable acidity (TA, between 0.12 and 0.16), total solids, microbial counts (ideally less than 50,000 per mL), and no positive beta-lactam antibiotics test results (Potter 2004). The delicate flavor of cottage cheese will not mask quality defects in the milk. Total solids are an important component of skim milk. Normal skim solids will vary from 8.8 to 9.2% (Potter 2004). Low solids results in fragile curd, so standardizing to between 9.0 and 10.0% total solids is indicated (Potter 2004). Skim milk may be fortified with extra-grade, low-heat, spray nonfat dry milk (NFDM) or evaporated milk to increase solids and cheese yield (Kosikowski and Mistry 1997a). About 5 kg of NFDM powder is needed to raise the solids 0.5% for each 1000 kg of skim milk (Potter 2004). The NFDM should be added prior to pasteurization, and three to four hours of rehydration prior to cheesemaking is recommended for optimum yield from NFDM (Potter 2004). Reconstituted skim milk may also be used to make cottage cheese, but drainage of curd may be slowed (Kosikowski and Mistry 1997a). If the total solids level of reconstituted or fortified milk is too high, the curd will be heavy, soggy, or unnatural, with high acidity, so care must be taken in optimizing total solids (Potter 2004).

26.2.1.2 Calcium. Although not used commonly in the cottage cheese industry, calcium chloride is permitted, at a legal maximum of 0.02%, to prevent soft fragile curd (Kosikowski and Mistry 1997a).

26.2.1.3 Cultures. Ideally, cottage cheese cultures should be mesophilic homofermentative lactic acid bacteria that do not produce large quantities of diacetyl or volatile fatty acids that will be lost in the whey during curd washing (Watrous 1997). Pure cultures of *Lactococcus lactis* subsp. *lactis* or *L. lactis* subsp. *cremoris*, or a mixture of these species, satisfy these criteria. Additionally, a starter culture that combines *L. lactis* and/or *L. cremoris* plus *Leuconostoc mesenteroides* subsp. *cremoris* is suitable, as long as large amounts of carbon dioxide are not produced (Watrous 1997). *L. lactis* subsp. *diacetylactis* is generally not satisfactory for cottage cheese (Watrous 1997).

Cultures are available in a variety of forms, including conventional or pH-controlled bulk starter, and direct vat set (DVS) culture. Selection of one variety or another is influenced by plant size, volume capacity, and production scheduling. In the case of conventional bulk starter, usage rate is typically 5–7% for a 4–5 h set (Potter 2004). Either internal or external pH control bulk starter cultures can also be used for cottage cheese production (Potter 2004). Because of the higher cell population than in a conventional starter, a usage rate of 1.5–2.5% of the skim volume is appropriate for a 4–5 h set; lower inocula are used for longer sets (Watrous 1997; Potter 2004). Direct vat set cultures,

either frozen or freeze-dried, are convenient and commonly used in the industry. They are more concentrated and more expensive than bulk starters. Usage rate varies from 0.20 to 0.25% (Waltrous 1997).

An alternative to the use of cultures in the production of cottage cheese, as a time-saving device, is direct acidification. Approved acids include food-grade phosphoric, citric, and hydrochloric acids (Koslikowski and Mistry 1997a). Glucono-δ-lactone may also be used (Koslkowski and Mistry 1997a). Cottage cheeses made in this fashion require the container to be labeled with "directly set" or "curd set by direct acidification" (Kosikowski and Mistry 1997a). Directly set cottage cheeses lack the unique and complex flavors imparted by cultures. Also, depending on economic conditions in the market, the ingredients used for direct acidification may prohibit their use due to their high cost. Typically, direct acidification may cost \$0.01–0.03/kg more.

26.2.1.4 Enzymes. Although cottage cheese is an acid-set cheese and can be made entirely without the use of coagulating enzymes, modern processing facilities use chymosin (rennet) or a coagulating enzyme produced from the fermentation of *Mucor meihei*, to reduce the processing time and increase yield. The resulting cheese is also sweeter and milder in flavor (Potter 2004).

Normal usage for single-strength rennet is 1–2 mL per 4550 L (small curd) or 4–6 mL per 4550 L (large curd). Double-strength rennet is not normally recommended for use in cottage cheese manufacture, because the small amount required increases error potential. Instead, many commercial cheese companies use a prediluted rennet labeled as cottage cheese coagulator. This product is used at a rate much higher than single-strength rennet – (40–60 mL per 4550 L (small curd) or 100–120 mL per 4550 L (large curd). This will vary based on the manufacturer and desired curd texture. Coagulant must be diluted 20 to 40 times to prevent localized coagulation during addition to the milk. Dilution should be done immediately before addition to milk, to prevent inactivation by chlorine in tap water. Soft sets are preferable to firm sets in the case of cottage cheese, so lower levels of chymosin or coagulant are recommended over high levels (Potter 2004).

26.2.1.5 Cream Dressing. Cottage cheese cream dressing may be made with sweet cream or sour cream. As with the skim milk, the cream must be of prime quality. Commonly, dressings contain a combination of any of the following: whole milk, heavy sweet cream or sour cream, salt, cultured buttermilk and/or skim milk powder, and, in most products, stabilizers. The stabilizer systems typically used consist of a combination of gums, starches, emulsifiers, and phosphates (locust bean gum, guar gum, carrageenan, starch, xanthan, lecithin, and sodium and potassium phosphates). The usage rate of stabilizers in cottage cheese dressing is usually less than 0.50%. The blend may also contain a preservative such as potassium sorbate to inhibit mold growth. Each ingredient is individually labeled on the finished cottage cheese container. Stabilizer systems help the dressing adhere to the curd surface and prevent defects such as "free cream". Overuse of stabilizers will cause the finished cheese to have a "slick" mouthfeel. The level of milkfat in the cream dressing for regular cottage cheese typically ranges between 9 and 11% (Potter 2004). The fat content of the dressing is standardized with the ratio of cream to curd, as described later. A typical cream dressing may consist of 10% milk fat, 11% MSNF, 0.35% stabilizer, and 2.25% salt, for a total solids content of 23.60% (Potter 2004). For such a composition, 18.4 kg of 40% fat cream, 65.3 kg of 3.5% fat milk,

3.7 kg of NFDM, 10.0 kg of cultured whole milk (3.5% fat), 2.25 kg salt, and 0.35 kg stabilizer would be used for each 100 kg of dressing (Potter 2004). The sweet cream dressing described typically results in a cottage cheese with pH between 5.2 and 5.3, which has a short shelf-life. Cultured buttermilk may be added to the dressing in quantities up to 10%, before pasteurization, to bring the pH to or below 5.0 for the finished cheese, which extends shelf-life (Potter 2004). The dressing pH may be as low as 5.9–6.1 prior to pasteurization, but anything lower may cause the dressing to precipitate during pasteurization.

The dressing is pasteurized at 75–82°C for 30 min by the vat method or 88°C for 32 s by the high temperature short time (HTST) method (Potter 2004), then cooled to 60°C for homogenization at 2000 psi (13.77 MPa) (first stage) and 500 psi (3 MPa) (second stage). The dressing should be held, refrigerated, for 20 h to 3 days before application to curds for viscosity to develop (Potter 2004). The high temperatures impart a pleasing cooked flavor and nearly sterilize the cream dressing for an extended shelf-life (Potter 2004).

Blanchette and others (1995) demonstrated that fermenting cottage cheese dressing with probiotic bacteria, *Bifidobacterium infantis*, has potential as a product for lactose maldigestive patients. In a follow-up study, Blanchette and others (1996) found that *B. infantis* introduced into fresh cheese exhibited vigorous metabolic activity during storage for 28 days. However, consumers preferred the control cottage cheese and the cottage cheese made with the dressing fermented to pH 5.5 to the cottage cheese with added dressing fermented to pH 4.5.

26.2.1.6 Water. One thing that must not be overlooked in the production of quality cottage cheese is a reliable source of filtered, chlorinated chilled water (Potter 2004). The wash water pH should be adjusted to 5.0 with food-grade phosphoric acid and contain 8–12 ppm chlorine. Conventional washing of the curd in the cheese vat will require approximately 2.5 times as much water volume as milk volume to properly cool the cheese curd prior to creaming. It is important to wash the curd after cooking to achieve two main goals:

1. Remove excess whey and
2. Cool the curd to stop the cooking process.

The final curd temperature after washing should be between 5 and 10°C. Automatic curd-washing equipment can reduce the wash water usage by as much as 50% to achieve the same results.

One aspect of rinse water that should be considered is the source of the water. Water from reservoirs can be of inconsistent quality if the watershed flora is predominately deciduous trees. For instance, autumn runoff from such forest floors will contain extracted organic materials that react with municipal chlorine added in municipal water systems and will yield harsh flavors.

26.2.2 The Process of Cottage Cheese Making

26.2.2.1 Sanitation. For any quality product to be produced, sanitation is of utmost importance. Cleaning and sanitizing all equipment, utensils, walls, floors, and drains is critical to maintaining safety, quality, and productivity. Poor sanitation practices will lead to poor quality or shortened shelf-life, delayed fermentation of vats (due to

bacteriophage contamination) at best, and at worst, public health issues. Stainless-steel equipment and utensils are recommended for cottage cheese manufacture for their ease in cleaning and sanitizing (Potter 2004). Chlorinated alkaline cleansers are effective at removal of organic soils, especially protein, making them most useful in a cheese plant. Acid cleansers are indicated for the removal of mineral soils. Use of chlorine sanitizers is recommended over iodine sanitizers because they are more effective against bacteriophages (Potter 2004). Manufacturers should always be consulted for determination as to which cleaning and sanitizing supplies and procedures are appropriate for a given plant.

26.2.2.2 Separation. A cottage cheese plant may elect to purchase pasteurized skim milk and cream or purchase raw whole milk and separate it into skim milk and cream. Most modern facilities are equipped with in-line separators. Milk is separated prior to fortification and/or pasteurization.

26.2.2.3 Pasteurization. Legally, the skim milk for cottage cheese must be pasteurized to kill pathogens and other undesirable microorganisms that may be present in the skim milk (Potter 2004). For best quality, the skim milk for cottage cheese manufacture should be pasteurized at the minimum allowable temperature: 62.8°C for 30 min or 71.7°C for 15 s (Watrous 1997). If heated at too high a temperature, a soft, high-moisture coagulum with high acidity will result (Watrous 1997; Potter 2004). For California-style cottage cheese, pasteurization and cooking temperatures are higher than for other styles (Kosikowski and Mistry 1997a).

26.2.2.4 Homogenization. Because skim milk is used, homogenization is optional. However, homogenization is highly recommended because it helps prevent agglutination (sedimentation) and fat separation in the cheese vat if the separator is not functioning properly (Potter 2004). Homogenization pressure of 500–1000 psi (3–7 MPa) in a single-stage homogenizer is recommended (Potter 2004).

26.2.2.5 Culture Preparation. As mentioned previously, the cottage cheese maker has options for culture selection. Bulk starter culture will need to be prepared at least one day in advance of cheesemaking. Commercial bulk starter media are available and primarily contain the following: sweet whey or whey solids, nonfat dry milk, yeast extract, phosphates, and trace minerals. This preblended media replaces conventional reconstituted nonfat milk starter media and offers the advantage of concentrated starter production using pH control and also bacteriophage control. As a result of the use of these media, starter room location and construction has become easier with the use of phage-inhibitory media as compared with conventional milk starter. These media allow starter to actually be grown in the same room as the cheese vats. Although the ideal condition is to have a separate starter room with its own air-handling system, cottage cheese manufacture is feasible without one. It is good practice to incorporate filtered positive-pressure air to the starter tank to help prevent airborne bacteriophage contamination during the processing and inoculation of the starter.

Bulk starter is prepared by reconstituting the commercial medium with water to various solids levels, ranging from 6.0 to 11.0%. The reconstituted medium is then pasteurized (vat method, 85°C for 30 min, or HTST method, 95–100°C for 30–120 s). After pasteurization the medium is cooled to 25°C and inoculated with a commercial mesophilic lactic acid culture. Incubation requires 12–16 h. During incubation, the pH of the starter is

maintained or controlled through the neutralization effect of buffers or neutralizers. This is accomplished with either internally blended phosphates and/or citrates or by externally added aqueous ammonium hydroxide. Neutralization allows the culture to continue fermentation and produce a more concentrated cell concentration per volume of starter versus conventional milk starter with no pH control. Once the starter is complete, it is cooled to 5°C and can be stored and used for up to 3 to 5 days.

26.2.2.6 Culture Addition. Generally, after pasteurization, milk is cooled to set temperature, directed into the cheese vat, and immediately cultured. However, current manufacturing processes also include the use of pasteurized-milk-holding tanks to allow for continuous availability of pasteurized skim milk. Milk is pasteurized and stored cool until it is needed. It is then pumped through a heating device (that is, a triple tube or plate heat exchanger), where it is tempered to 32°C and sent to the cheese vat. This helps prevent downtime or scheduling problems with the HTST unit when vats are refilled for multiple rounds per day. Care should be taken to avoid excessive air incorporation into the milk, as cultures are facultatively anaerobic or microaerophilic. Ideal temperatures for mesophilic lactic cultures used in cottage cheese vary between 21 and 33°C, with 30–33°C being most commonly used (Kosikowski and Mistry 1997b; Watrous 1997). Selection of temperature depends on the manufacturing technique (Table 26.1). If milk is to be cultured in the evening and allowed to ferment until morning (12–16 h setting time), a temperature of 21–24°C is appropriate. If milk is to be cultured in the morning and the process is to be carried out in an 8-h period or less, the setting temperature should be 30–33°C (Watrous 1997).

In the case of conventional cottage cheese making, bulk starter should be added to the vat once the vat is at least one-half full, because the low pH (<5.0) of the starter could cause localized coagulation if added to a low volume of milk (Potter 2004). If using pH-controlled bulk starter or DVS culture, the starter can be added concurrently as the cheese vat is filled (Potter 2004).

26.2.2.7 Enzyme Addition. Diluted chymosin may be introduced into cultured milk immediately after the vat is full, or after a short incubation period (less than 30 min) (Kosikowski and Mistry 1997b). The chymosin should be mixed into the cultured milk for approximately 2 min, so it is well distributed, then the milk should remain quiescent, covered, for approximately 5 h, until the pH of the curd is 4.7–4.6 (Kosikowski and Mistry 1997b; Potter 2004). It is important that all disturbing vibrations be minimized during the setting period, because cottage cheese curd is very fragile.

26.2.2.8 Cutting. Regardless of enzyme use, cottage cheese coagulum must be cut when the curd is "ready". Cutting the curd at the proper pH is the single most important

TABLE 26.1 Processing Parameters of Different Styles of Cottage Cheese.

Process Stage	Long-Set	Intermediate-Set	Short-Set
Ripening prior to cut	12–16 h	8 h	5 h
Temperature of milk set	22°C	26.5°C	32°C
Starter addition			
Conventional	1%	2%	5%
pH Contolled	0.5%	1%	2%

factor in producing a high-quality cottage cheese, and each plant should find the optimum cutting pH for the operation then adhere to it for a consistent product (Potter 2004). Although titratable acidity (TA) is utilized widely in the production of Cheddar and other cheeses, pH is more appropriate for monitoring cheese curd formation in cottage cheese production (Potter 2004). The TA of whey at pH 4.6 will be approximately 0.42–0.60, but varies according to the solids content of the skim milk (Watrous 1997; Potter 2004). At its isoelectric point, casein is least soluble and exerts its lowest moisture-binding-capacity (Watrous 1997). At this point, cutting, stirring, and heating stimulates the curd to release moisture (Watrous 1997). Curds should be cut at a pH of 4.65–4.70 for small curd and 4.70–4.75 for large curd (Watrous 1997; Potter 2004). Cutting at pH above 4.7 typically results in a curd that is lumpy and tough, but cutting at a pH below 4.5 results in a curd that is soft and fragile (Potter 2004). Thus, each plant must monitor pH with reliable equipment, determine the optimum pH for cut, and aim for consistency.

In addition, it is good practice to do a visual check on the appearance of the coagulum by taking a sample using a "sample straw", which is used for milk sampling in bulk trucks. The test involves inserting a long sanitized straw into the curd, plugging the top of the straw with finger, and removing the loaded straw. The straw should be placed on a flat surface and slowly drag backwards as the finger is removed from the straw opening at the top. The curd is ready when it snakes across the surface, holds structure, and yields only clear whey. This method should be used in conjunction with a pH meter and not as a standalone test. Its purpose is to confirm that the coagulum firmness matches the cut pH. Information gained from this test should be used to make adjustments in the amount of coagulant to be used in future vats.

For cottage cheese curds, the coagulum should be cut with 1.3-, 1.6-, or 1.9-cm knives for small to intermediate to large curd sizes (Kosikowski and Mistry 1997b). Consistency in cutting is critical, because overly large or excessively small curds result in inconsistent curd size, moisture content, and yield losses.

26.2.2.9 Healing. Because cottage cheese curds are extremely fragile, curds must be allowed to heal, undisturbed, for 15–30 min. (Kosikowski and Mistry 1997b). Healing allows a skin to form on the surface of each curd, minimizing damage during the subsequent cooking process, which could affect cheese yield. During the healing process, whey is expelled, shrinking and firming the curd and permitting easier agitation of the curd during the cooking process.

26.2.2.10 Cooking. At the beginning of the cooking process, the curds are still fragile. A paddle should be manually used to gently and methodically ease curds apart and away from the vat walls. After the curds drop about 5 cm from the whey surface, warm water (about 5 cm) may be added to the cut curd in the vat before stirring to raise the temperature slightly (Kosikowski and Mistry 1997b; Watrous 1997). The vat jacket temperature, which is typically 10–15°C higher than the curd–whey mixture, should be raised gradually to raise the curds and whey temperature to 53°C (in winter) or 56°C (in summer), slowly (Potter 2004). A steady 1.7°C rise every 10 min is recommended (Kosikowski and Mistry 1997b). Curds may be stirred either manually or mechanically after the initial stages (30 min) of cooking in a manner that prevents both matting (too slow) and breakage (too vigorous) (Potter 2004). Improvements in vat design and agitation methods have made it unnecessary to use manual agitation. These new designs utilize a vertical stir motion as opposed to a circular one. As a result, the

curd does not go unstirred in the vat corners and the curds experience a more gentle continuous stirring action. The heating process has also been improved and heat is now added outside the cheese vat. Surface whey is drawn out of the vat and heated either by direct steam injection or tubular heat exchanger and then pumped back into the vat on the top surface. This allows the heat to be concentrated initially in the whey portion and the curd is then gently stirred up into the hot whey. A more uniform heating of the curd results with this process, as jacket heating can create localized hot spots, resulting in matting or excessive fines due to overstirring.

No acid development occurs after the vat contents reach 40°C (Watrous 1997), and starter bacteria are essentially all killed after a temperature of 53°C is attained (Potter 2004). Ideal curd firmness is attained from about 100–120 min after the initial temperature rise (Kosikowski and Mistry 1997b; Potter 2004).

Commonly today, phosphoric acid is used as an aid in cooking because it creates an imbalance between curd and whey acid (Potter 2004). Food-grade 75% phosphoric acid is added to the curd–whey mixture just after healing and initial gentle mixing (Potter 2004). Enough acid is added to reduce the pH of the vat to 4.4–4.5 when the vat temperature is 38°C, about 50–75 mL per 455 L of skim milk (Potter 2004). The acid should be diluted a minimum of 20 to 40 times with clean water before adding to the vat. Always follow safety precautions when handling phosphoric acid.

Curds can be tested for firmness after the optimum temperature is attained. Curds can be dropped from waist level (roughly 75 cm) and observed for bounce. If curds spatter instead of bounce, sufficient firmness has not yet been reached and temperature should be held for another 10–30 min. Alternatively, a clump of curds can be taken in the hand and rinsed. If they are ready for washing after squeezing, the curds should bounce back to their original shape. Both of these methods are very subjective and require a great deal of experience. Unfortunately, there is not a consistent mechanical method of analysis that will give a quick determination of curd firmness at the end of the cooking time. If the curd is too soft after cookout, the curd was cut too late and the pH at cutting should be raised in the next vat (Potter 2004). If the curd is too firm after cookout, the cut was likely too early and the opposite adjustment is indicated.

26.2.2.11 Washing. The purpose of washing the cottage cheese curds is to remove whey and cool the curd. The whey will impart an acid flavor. High-acid cottage cheese is not as desirable to most consumers as mildly acidic cottage cheese. Wash-water pH is important to cheese quality. To help minimize the growth of psychrotrophic organisms, the wash-water pH should be adjusted to a pH of 5.0 and should contain 8–12 ppm of chlorine. This pH closely matches that of the finished cheese. Wash water should be cooled to below 7°C.

Washing is accomplished using two methods: Vat and automated. To begin the vat washing process, agitation of curds is stopped and the curds are allowed to settle at the bottom of the vat. A paddle should be used to pull the curds back about 30 cm from the exit port to make way for the insertion of an inside-the-vat strainer into the exit port. Enough whey should be drained until about 10–12 cm depth of piled curd begins to show through the whey. At this time, treated wash water should be added to the rear of the vat. Enough water should be added such that the whey exiting the drain reaches approximately 50°C. At this time, the vat drain port is closed, the strainer removed, and the wash–water–whey–curd mixture agitated for 5–10 min. Rinse water is continually added until the curd temperature drops from about 53°C to 27°C.

After a temperature of about 27°C is reached, the strainer should be returned to the vat as before, and the wash-water–whey drained completely. The exit port is then closed, chilled treated water is added to the vat to the level of the previous wash-water–whey–curd level, the strainer is removed, and agitation is resumed for 5–10 min. At this point, the wash-water–curd temperature should be close to 4°C. When a single vat is used for the entire cottage cheese production, washing generally takes place in three cycles (Bylund 1995). The washings bring the temperature of the curd down to 30, 16, and 4°C, respectively (Bylund 1995). For the final drain, curds should be moved to the rear of the vat, as usual. As the wash water is drained, the curds should be trenched, or piled on two sides of the vat, and allowed to drain naturally. Complete draining takes approximately 45 min. Cream dressing must be ready for the creaming process so curds are not allowed to overdrain.

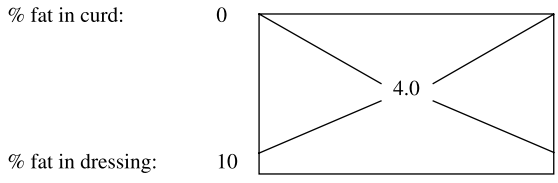
For automated curd washing, after the cook process is completed, the curd and whey mixture is pumped to a whey drainer or curd washer. The whey is separated from the curd either by a nylon or stainless-steel mesh screen and the curd is dispersed in cool wash water. Depending on the style of automated washing, it will be accomplished by using either a vertical washing tower or a horizontal washing tank. Each will result in the curd being cooled to approximately 7–10°C. The advantage of using the automated cooling is reduction in wash-water usage and also increased production and economic efficiency by allowing the washing process to occur outside of the cheese vat. The process frees up the cheese vat to be reset. The advantage of the vat washing method is better curd identity. After washing, the curd is again transferred, via pumping, to a curd drainer, where the water is removed and the curd enters a creaming vessel.

26.2.2.12 Creaming. For proper meaty cottage cheese curd texture and pleasing flavor, curds should be creamed immediately after proper draining (Potter 2004). The most common ratio of curd to cream dressing is 60:40, but it varies among manufacturers. This translates into approximately 10 L of dressing for each 100 L of skim milk set (Potter 2004). Application of pasteurized, homogenized cream dressing with 11% fat at the rate of 56 kg per 100 kg curd with normal moisture results in a highly satisfactory product (Potter 2004). If a curd with lower moisture content than the target value is produced, a lower fat (10%) dressing of higher moisture content may be used (Potter 2004).

The amount of cream dressing and curd to combine for the appropriate fat content final product can be determined by use of the "Pearson Square" method, shown in Figure 26.1. For example, if there are 100 kg of curd to be creamed and 10% fat cream dressing is available, the amount of cream necessary to give a fat content of 4% in the finished product is 66.7 kg.

As with the washing process, there are two basic methods used for creaming the curd. The first is the vat method, where dressing is added to the curd that has been rinsed and drained in the vat. This method requires skill and experience in that most cheese vats will not be on load cells and the exact amount of curd in the vat can vary from day to day. This makes it more difficult to determine the amount of cream dressing to add. Through years of experience, cheesemakers will become expert at estimating the appropriate amount of dressing needed based on appearance. Once the dressing is added, it is either stirred in by hand or by mechanical agitation.

The second method of creaming is used in conjunction with the automated washing system. Once the curd is washed it is transferred to a creaming tank. The tanks can either be vertical or horizontal depending on the type of mixing agitator. These tanks



The difference between opposite corners is calculated to determine number of parts, or component ratios. In the current example, to obtain 4% fat product, the curd and cream would be mixed at the rate of 6 parts curd and 4 parts cream dressing.

$6 / 4 = 100 / x$

$6x = 400$

$x = 66.7$ kg of 10% fat dressing, needed for 100 kg of dry curd

Proof:

66.7 kg	10% dressing contains	6.67 kg fat
100 kg	0% curd contains	0.00 kg fat
166.7 kg	4% creamed curd contains	6.67 kg fat

Figure 26.1 *Pearson square determination of cottage cheese creaming ratio.*

will be placed on load cells so the exact weight in each can be measured. Once the desired creaming ratio is calculated, the dressing is metered into the creaming tank at the same time the curd is entering. This aids in the mixing of the cream and curd and allows the operator to achieve the exact ratios of curd and cream entering the creamer.

26.2.2.13 Packaging and Shelf-Life. A good package should provide structure, visual appeal, and most importantly, protect its contents against light, oxygen, loss of moisture and aroma, and contamination (Mortensen and others 2004; Zhao 2004). Packaging materials used for fresh cheeses, including cottage cheese, may include polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), and polyethylene (PE)/polyamide (PA) combinations, forming oxygen barriers in modified atmosphere packaged products (Mortensen and others 2004; Zhao 2004). However, vacuum-formed PS tubs are now used almost exclusively for packaging of cottage cheese because they protect the cream dressing from fat absorption and breakage (Zhao 2004). The PS tubs can also be pigmented with titanium dioxide, which provides improved light-barrier properties (Zhao 2004). A shrink-film of PP under the outer lid of the cottage cheese is effective as a tamper-evident seal, and also serves as a moisture barrier (Zhao 2004). Vinyl shrink bands are an alternative to the more costly PP seal (Zhao 2004).

Cottage-cheese shelf-life is typically about 21–28 days at 7°C (Chen and Hotchkiss 1991). The greatest enemies limiting shelf-life are microorganisms including coliforms, yeasts, molds, and psychrophiles. Acceptable initial ranges for these microorganisms are 0–10 per gram for coliforms, yeasts and molds, and up to 100 per gram for phychrophiles (Potter 2004). Contamination of cottage cheese with psychrotrophic Gram-negative bacteria such as *Pseudomonas*, *Alcaligenes*, *Proteus*, *Aerobacter*, and *Aeromonas* leads to undesirable flavors and slimy curds (Chen and Hotchkiss 1991). Yeasts and molds (*Geotrichum*, *Penicillium*, *Mucor*, *Alternaria*) cause flavor, textural, and visual spoilage (Chen and Hotchkiss 1991). Sorbate may be added to cottage cheese at a concentration of 0.25% to extend shelf-life by inhibiting yeasts, molds, and some spore-forming microorganisms, but sorbate contributes a bitter off-flavor to cottage cheese.

Some manufacturers may extend the shelf-life to 60 days with carefully controlled packaging conditions (Chen and Hotchkiss 1991). Kosikowski and Brown (1973) flushed the headspace of cottage cheese with CO_2 or N_2 and noted inhibition of yeasts, molds, and psychrotrophic bacteria. However, it was not clear if the benefit was from the gases themselves or simply the removal of O_2. Chen and Hotchkiss (1991) demonstrated that dissolved CO_2 effectively inhibits the growth of Gram-negative bacteria in creamed cottage cheese packaged in high-barrier containers. The authors did not conduct sensory evaluation. Moir and others (1993) inoculated creamed cottage cheese, pH 4.9, with pseudomonads. An atmosphere of 40% (v/v) CO_2 inhibited the growth of pseudomonads by increasing lag times and reducing growth rates. Inhibition by CO_2 was greater at the surface than in the depth of the cheese. The flavor and pH of the cheese were not affected by the CO_2. Later, Maniar and others (1994) demonstrated that cottage cheese packaged in barrier containers under modified atmospheres (100% CO_2, 75% CO_2, 25% N_2, 100% N_2) exhibited no change in psychrotrophic and lactic acid bacteria counts and exhibited satisfactory sensory characteristics after 28 days of storage at 4°C. Cheese flushed with 100% CO_2 received highest sensory scores (Maniar and others 1994). More recently, Mannheim (1996) found that an optimum headspace of about 25% (v/v) in 250 mL packages, flushed with pure CO_2, extended shelf-life of cottage cheese at 8°C by about 150%, without altering sensory properties or yielding any other negative effect. The authors stressed the importance of using high-barrier packaging material in order to maintain the proper CO_2 level in the headspace and cause its dissolution into the product (Mannheim 1996).

26.3 EVALUATION OF COTTAGE CHEESE

High-quality cottage cheese should have a clean, slightly acidic flavor with a slight buttery (diacetyl) aroma and a creamy flavor note. There should be no particular aftertaste and only a sufficiently salty taste to bring out desirable flavor (Bodyfelt and others 1988). Cottage cheese body should be somewhat like cooked chicken breast (meaty), but not overly rubbery or tough when first chewed or pressed by the tongue on the roof of the mouth. The texture should be relatively smooth throughout. The evaluator should be able to see and feel distinct curd particles that are uniform in shape and size. The creamed cheese should be white, and exhibit a moderate gloss or sheen, and the cream dressing should cling to curd particles (Fig. 26.2). Common attributes of cottage cheese, along with descriptions and probable causes for deviation from ideal are included in Tables 26.2, 26.3, and 26.4 and Figures 26.3, 26.4, and 26.5.

Figure 26.2 *Appearance of the creamed cheese.*

TABLE 26.2 Common Flavor Attributes in Cottage Cheese, Identification, and Their Probable Causes (3).

Flavor	Identification	Probable Cause
Bitter	Sometimes perceived as throbbing/piercing Sensation perceived at back of tongue	Poor quality milk Low salt Proteolytic starter culture strains Microbial contaminants Excessive acidity Plant sanitation issues
Cooked	Cooked cottage cheese may have an eggy, charred or caramel aroma or flavor	Over-heating of cream dressing Cottage cheese cream dressing is commonly cooked; not undesirable unless excessive
Feed	Grassy flavor	Feeding of strong flavored feeds Feeding of cattle too close to milking
Fruity/fermented	Sweet–like pineapple, apples or bananas	Certain strains of *L. lactis* Low acidity Low salt level Poor milk quality
Flat/lacks flavor	Lacks flavor and aroma components Lacks diacetyl or creamy flavor Reminiscient of uncreamed curd	Lack of acid production Low fat dressing

(*Continued*)

TABLE 26.2 *Continued.*

Flavor	Identification	Probable Cause
Foreign	Unlike any other off-flavor that might be anticipated in cottage cheese	Chemical sanitizers Unknown contaminant
High acid	Excessive acid taste Unbalanced acid taste	Development of excessive lactic acid Use of too much starter culture Use of high-acid milk Improper whey expulsion from curd Low salt level
High diacetyl	Diacetyl is out of balance May be associated with bitter aftertaste Harsh aroma	Excessive diacetyl production by starters Excessive diacetyl addition to dressing Imbalance among diacetyl and other flavor compounds
High salt	Unbalanced sharp or piercing taste sensation	High salt concentration in cream dressing
Lacks fine flavor	Cottage cheese smells or tastes like yogurt, is harsh, or out of balance	Improper selection of starter cultures Nonstarter lactic acid bacteria contamination
Metallic	Astringent, rusty nail-like flavor	Contamination with copper or other reactive metal
Oxidized	Paperboard/cardboard Burnt hair aroma/ flavor	Use of oxidized milk in cheesemaking Use of oxidized cream in dressing
Rancid	Butyric, caproic, caprylic, capric acids Soapy Baby vomit aroma Romano cheese aroma/flavor	Milk lipase activity Microbial lipase activity (from contaminants) Accidental homogenization of raw milk Late lactation or mastitic milk
Sweet	Sweet taste	Associated with excessive use of stabilizers in dressing
Unclean	Unpleasant off-flavor lingers	Microbial contamination Poor quality off-flavored or old milk/cream Aged cheese
Whey	Combination of acid, bitter, fermented Aftertaste does not linger like unclean	Poor whey expulsion from curd Improper curd washing techniques
Yeasty	Ethanol aroma (bread, beer)	Development of ethanol flavors by yeast Poor packaging procedures

Regional differences do exist in flavor and appearance of cottage cheese within the United States. Historically, the midwest and northwest style of curd will tend be to firmer and have a wetter appearance from the dressing, but east and west coast cottage cheese tends to have a softer curd and drier appearance with a lesser degree of curd identity. As with other cheeses, certain identified defects can be considered attributes depending on consumer preference and tastes.

TABLE 26.3 Common Body and Texture Attributes in Cottage Cheese, Identification, and Their Probable Causes (3).

Body/Texture	Identification	Probable Cause
Firm/rubbery	Resistant to compression by tongue	Over-cooking of curd Excessive acid production Improper dressing formulation
Mealy/grainy	Grainy (like corn meal) breakdown of curd during mastication	Excessive acid production Formation of salt complexes
Overstabilized	Product seems slick Curds slip between teeth Dressing may appear thick	Excessive use of stabilizers in cream dressing
Pasty	Sticky when masticating Gel-like, doughy Often associated with weak/soft	High moisture retained by curd
Weak/soft	Mushy when pressed to roof of mouth	High moisture in cheese Improper cooking of curd Improper dressing formulation

TABLE 26.4 Common Appearance and Color Attributes in Cottage Cheese, Identification, and Their Probable Causes (3).

Body/Texture	Identification	Probable Cause
Free cream	Cream dressing flows away from and does not adhere to curds	Inadequate use of stabilizers Low-solids cream dressing
Free whey	Clear yellow fluid between curd particles and separated from curd	Inadequate washing steps Low-solids cream dressing
Gassy	Excessive gas bubbles	Contamination of cheese with CO_2–forming microorganisms (or gas flush)
Lacks cream	Curd particles appear dry Curd particles lack gloss	Improper creaming ratio Excessive stabilization
Matted	Masses of curd particles are clumped	Improper curd stirring in vat
Shattered curd	High variability in shape and size of curd particles Extremely common attribute	Mechanical breakage of curd particles

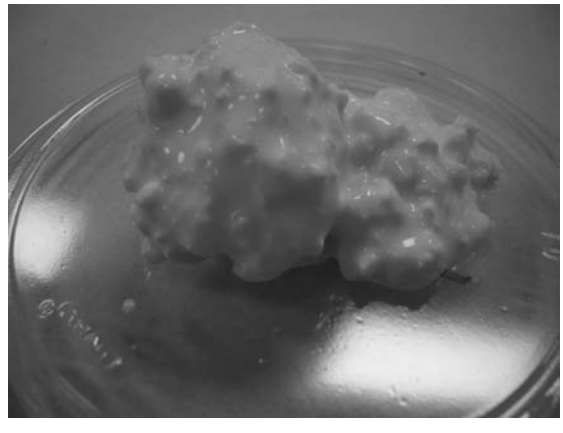


Figure 26.3 *Creamed cottage cheese exhibiting mathed curds.*

Figure 26.4 *Creamed cottage cheese exhibiting a lack of cream dressing.*

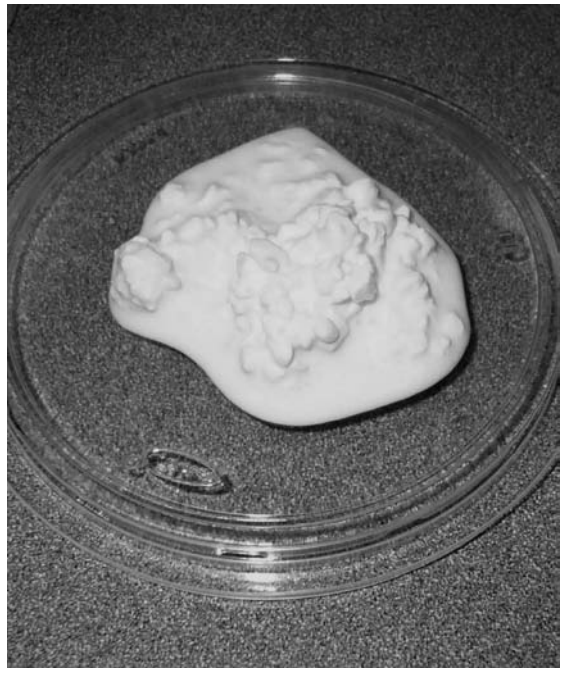


Figure 26.5 *Creamed cottage cheese with excess dressing or "free cream" defect.*

26.4 SUMMARY

Cottage cheese is a very delicate and difficult product to make on a consistent basis. To make a high-quality and consistent product, the processing steps reviewed must be adhered to and refined for each manufacturer. It is essential to not only have cheesemakers know the "Hows" of making cottage cheese, but to also understand the "Whys" in the processing steps. As dairy companies continue to consolidate and increase in size and

distribution area, regional varieties of cottage cheese are disappearing. It will be important for dairy companies to establish themselves with quality products in these new markets to maintain and provide growth in cottage cheese sales.

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27

Cheese Varieties Made by Direct Acidification of Hot Milk

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27.1 Ricotta Cheese 638
27.1.1 Manufacturing Procedure 638
27.2 Chhana 639
27.2.1 Manufacturing Procedure 639
27.2.2 Rasogolla 640
27.2.2.1 Cooking Medium 641
27.2.2.2 Sugar Syrup 641
27.2.2.3 Yield 642
27.2.2.4 Packaging 642
27.2.2.5 Rasmalai 643
27.2.2.6 Rajbhog 643
27.2.3 Sandesh 643
27.3 Paneer 645
27.3.1 Manufacturing Procedure 645
27.3.1.1 Milk Standardization 645
27.3.1.2 Heat Treatment 645
27.3.1.3 Curd Formation 645
27.3.1.4 Whey Drainage 646
27.3.1.5 Hooping 646
27.3.1.6 Pressing 646
27.3.1.7 Cooling 646

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

27.3.1.8 Packaging 646
27.3.1.9 Yield 647
27.3.1.10 Defects 647
27.3.1.11 Physico-Chemical Changes During Paneer Manufacture 647
27.3.1.12 Microstructure of Paneer 648
27.4 Latin American White Cheeses 648
27.4.1 Manufacturing Procedure 649
References 650

Cheese, in general, refers to conversion of vital milk constituents from fluid to semisolid or solid form. A crucial step in cheesemaking is the coagulation of milk, resulting in curd formation. This facilitates consolidation to the solid form of most proteins and fat along with the important mineral and vitamin fractions of milk. In most cheeses, curd formation is carried out with or without fermentation by application of coagulating enzymes isolated from various biological sources. However, certain cheese varieties do not involve the use of enzymes, and the milk coagulation is triggered by an acid either generated in situ by an added culture or by direct addition to hot milk.

Figure 27.1 shows classifications of acid-coagulated cheeses. They are separated into two types according to the coagulating procedure used. In the first type, coagulation of milk at ambient temperatures is accomplished using lactic acid produced as a result of fermentation with lactic acid bacteria. Cottage cheese, quark, and cream cheese are examples of this first type of acid coagulated cheeses, and are discussed elsewhere in this book.

In the second type (directly acidified cheeses), the curd is formed by direct acidification of hot milk with food-grade acids. In general, the directly acidified cheeses are consumed in fresh or unripened form. More recently, their shelf-life has been extended by sanitary production and packaging practices to fulfill the needs of modern marketing.

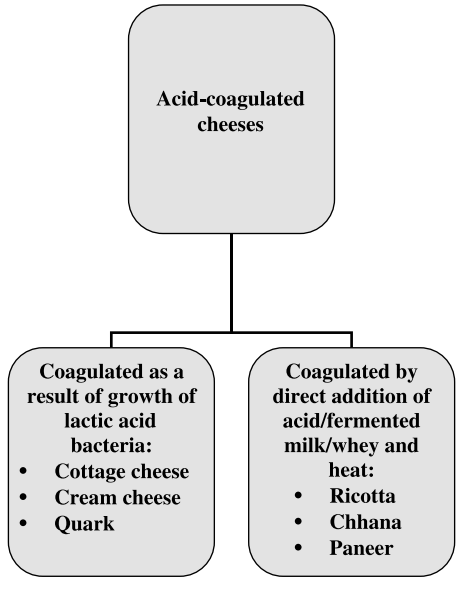

Figure 27.1 *Classifications of acid-coagulated cheeses.*

TABLE 27.1 Cheeses Made by Direct Acidification in Various Regions of the World.

Name	Country/Region	Type of Milk	Coagulants
Armavir	West Caucasus	Sheep	Sour buttermilk
Cecil	Armenia	Cow/sheep	Sour milk
Chhana	India/Bangladesh	Cow/buffalo	Lime/lemon juice/ citric acid/citric whey
Gruau de Montagne	France	Cow (whey)	Vinegar
Hudelziger/mascarpone	Switzerland	Cow (whey)	Vinegar
Kareish	Egypt	Buffalo/cow	Sour milk
Kesong Puti	Philippines	Buffalo	Vinegar
Paneer	India/Pakistan	Buffalo/cow	Citric acid/vinegar/ lime juice
Queso blanco	Latin America/ Central America/ Caribbean	Cow	Vinegar/fruit juices
Recuit/broccio/serac/ majette/ceracee	Italy/Europe	Cow (whey)	Vinegar
Requeijao	Brazil	Cow	Sour milk
Ricotta	Italy	Cow (whey–milk blend)	Vinegar
Ziger/schottenziger	Germany, Yugoslavia	Cow (whey)	Vinegar

White, unripened cheeses derived from milk coagulation with acid under sub-boiling temperatures are known by various names in different parts of the world (Table 27.1). In spite of wide differences in their manufacturing processes, these cheeses belong to the same group. They are made from whole milk, low-fat milk, skim milk, cream, whey, or their mixtures. Milk of various species of animals may even be utilized in production of these cheeses. Depending upon its origin, curdling of hot milk may be carried out with vinegar, lactic acid, calcium lactate, lime/lemon juice, acid whey, or yogurt/fermented milk. A direct acidification procedure using citric or acetic acid has been successfully applied to produce mozzarella and pizza cheese. Table 27.2 gives approximate chemical compositions of acid-coagulated cheeses.

Cheese varieties from directly acidified milk provide a substantial proportion of the required human intake of energy and protein, and significant amounts of essential nutrients

TABLE 27.2 Approximate Composition of Directly Acidified Cheese Varieties in Comparison with Cheddar Cheese.

Cheese	pH	% Moisture	% Fat	% Protein	% Lactose	% Ash	% Salt
Ricotta (whole milk)	5.8	72	13	11	2.9	1.0	0.5 max
Ricotta (part skim milk)	5.8	74	8	12	3.2	1.1	0.5 max
Ricottone	–	82	0.5	19	3.3	–	0.5 max
Mascarpone	5.0	46	47	4.5	–	–	0
Chhana (cow milk)	5.7	53.4	24.8	17.4	2.2	2.1	0
Chhana (buffalo milk)	5.4	51.7	27.0	14.4	2.3	1.9	0
Paneer (buffalo milk)	5.8	51.3	25–27	17.3	2.3	1.9	0
Latin American white/ Hispanic cheese	5.2–5.7	48.0	15–20	24.8	2.2	1.9	2.3
Queso blanco	5.2	55.0	15–27	23.0	2.5	1.9	2.5
Cheddar	5.6	37.0	32.2	25.0	0	1.2	1.8

Sources: Kosikowski and Mistry (1997), USDA (1998), Lucey (2000), and Chandan (2002).

such as calcium, riboflavin, magnesium, and vitamin B_{12}. High in protein and minerals but low in lactose, these cheeses offer many nutritional advantages including balancing a protein-deficient diet. As consumers are familiar with their flavor and textural attributes, no cultural barrier exists to develop or modify dietary patterns by incorporating these cheeses.

From the standpoint of commercial significance, ricotta, chhana, paneer, and Latin American white (LAW) cheeses will be discussed in this article.

27.1 RICOTTA CHEESE

Like cottage cheese, ricotta cheese is a high-moisture cheese. Ricotta is not a pressed curd type of cheese. Its composition varies depending on whether it is made exclusively from whey or from a blend of whey and milk. Originally, ricotta was produced from whey derived from mozzarella or provolone cheese production. Ricotta is now prepared from whole milk with or without addition of whey. When made from a blend of 95% sweet whey and 5% milk, ricotta contains 68–73% moisture, 16% protein, 4–10% fat, and 4% lactose.

Ricotta has a bland to slightly cooked but pleasing flavor. Its texture is soft and creamy. It is consumed as such as a spread and may be used as a replacement for cream cheese or sour cream in dips. It is basically a nonmelting cheese. Its major use is in cooking of Italian cuisine (for example, lasagna and ravioli) and confectionery.

27.1.1 Manufacturing Procedure

Most of the ricotta production is confined to a batch process. Traditionally, open kettles are used. Heating may be direct or steam jackets may provide heat transfer. Sweet whey from Italian cheese manufacture is suitable as long as its pH is 6.2 or higher. It is common to blend 10–25% milk to neutralize acid in the whey, enhance yield and curd cohesiveness. The mixture is heated in a kettle to 82–93°C, followed by the addition of a food-grade acid such as lactic, acetic, or citric acids in quantity enough to drop the pH of the mixture to 5.9–6.1. In some plants, cultured milk/whey may be used as a source of lactic acid. This pH range is crucial to maintain the sweet flavor of the cheese. As a result of heat and acidity, the proteins denature and the foam-type curd ascends to the surface. The mixture is held for 15–20 min, after which the curd is dipped with a perforated ladle and collected in a muslin bag. The bag is allowed to drip and cool in a cold room. Alternatively, the curd is drained in perforated stainless hoops and allowed to dry. The curd is soft, fragile, and grainy, and may be pressed slightly to achieve cohesiveness. The yield of ricotta is low, around 5–6%, if no milk is mixed with whey. Mechanized production for continuous manufacture of ricotta cheese involves adjustment of the pH of the whey-milk mixture to 6.9–7.1 with caustic soda, heating the blend to 88–92°C, and injecting an appropriate quantity of salt and acid in-line. Again, the target pH is 5.3–5.5. The hot acidified whey-milk blend is pumped into the bottom of a V-shaped vat and the resulting curd is mechanically collected from the top of the vat into a nylon mesh for the curd to drain. It is transferred to perforated hoops, cooled, and hot packaged for sale.

The ricottone cheese process is similar to that for ricotta, where sweet whey is blended with whole milk, skim milk, or buttermilk. The pH of the blend is adjusted to 6.1–6.2 with a starter culture or edible acid. Ricottone curd is pressed and dried for 4 weeks at 21°C to obtain dry ricotta cheese, a grating type of product.

Another variant of ricotta is mascarpone, which is made from cream (25–35% fat) by direct addition of citric or acetic acid to pH 5.0. Following drainage of whey in bags made of cloth, the cheese may be slightly salted, whipped, and formed into a cylindrical shape. Mascarpone has a creamier texture and richer flavor in comparison to ricotta. Unsalted mascarpone is used in the preparation of cakes and desserts like tiramisu.

Impastata is a starting material for pastry. It is made in a similar way to ricotta, but the curds are allowed to sink to the bottom by gentle stirring. The hot curd is drier than ricotta cheese. It is then ground into smooth dough-like material for use in confectionery.

The application of ultrafiltration technology to ricotta cheese has resulted in process improvements.

27.2 CHHANA

Chhana is a ricotta-like product in India, which is obtained from hot milk by direct acidification. According to the Indian Pure Foods Act, chhana is defined as a product obtained from cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid, or citric acid; it should contain not more than 70% moisture, and the milk fat content should not be less then 50% of the dry matter. Skim milk chhana is the product obtained from cow or buffalo skim milk by precipitation with sour milk, lactic acid, or citric acid; it should not contain more than 70% moisture, and the milk fat content of the product should not exceed 13.0% of the dry matter.

27.2.1 Manufacturing Procedure

Production of Chhana essentially involves precipitation of casein along with fat and entrapped water-soluble components of milk (lactose, whey proteins, minerals, vitamins) by the addition of an acidulant to milk at near-boiling temperatures followed by removal of whey from the curd. Chhana is made by a batch process, but a continuous process may be adapted from the continuous process for ricotta, as described above. Figure 27.2 illustrates the sequence of steps in the manufacture of chhana. The figure also shows the relationship between chhana, paneer, and LAW cheese.

The texture of chhana varies from smooth and pasty to crumbly. It is an intermediate starting material for the preparation of indigenous Indian dairy desserts and confectionery products. The preferred milk for channa-making is cow's milk. In chhana-making, pressing of the curd is restricted to that obtained by gravity after draining of the whey in a cheesecloth/muslin bag. Longer draining time gives a hard product. Depending on its end use, chhana may be soft or hard. For example, for the confection rasogolla, soft chhana is needed, but the confection sandesh requires the hard variety. These two confections utilize the majority of chhana production, and optimum functionality is required in each case.

Chhana, like ricotta is made in kettles. Cow milk is brought to near boiling and an appropriate acidulant is quickly added to hot milk to bring the pH of the mixture to 5.4. The curd settles to the bottom and whey is removed by filtration through a strainer lined with cheesecloth. The curd is cooled in running tap water. Chhana is then utilized to make sweetmeats and other Indian confections. The coagulants used are lime or lemon juice, vinegar, citric acid, lactic acid, fermented milk, and whey. A solution of citric acid (0.5–1.5%) or lactic acid (1–2%) is appropriate to lower the pH of hot milk

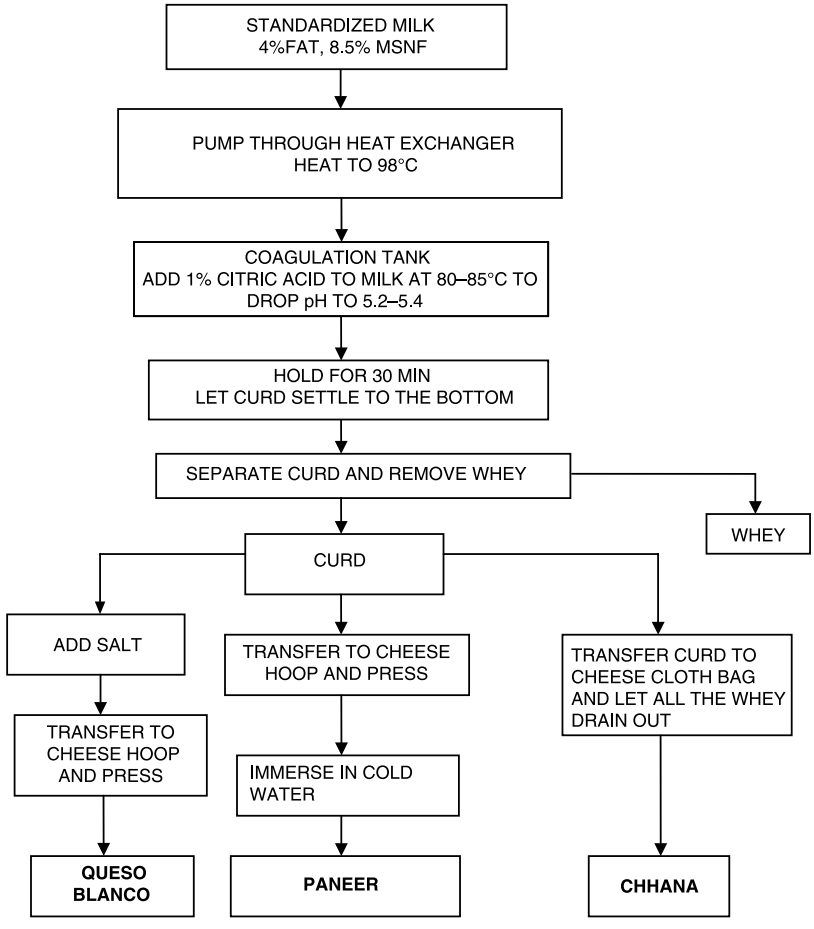

Figure 27.2 *Steps in the manufacture of chhana, paneer, and queso blanco.*

to 5.4. An interaction between the temperature of coagulation and final pH is observed in terms of obtaining the best yield and quality of chhana. For cow milk a temperature of 80°C at pH 5.4 is optimum, but for buffalo milk, 70°C at pH 5.7 is desirable. A higher coagulation temperature imparts graininess and hardness to the texture, and lower temperatures result in sticky chhana, which drains significantly slowly. Chhana is an extremely perishable food product, and at ambient temperatures, its quality deteriorates within a day or two. Therefore, soon after production, it must be used. Under refrigeration, the shelf-life is extended to six days. Two important confections based on chhana or rasogolla and sandesh.

27.2.2 Rasogolla

Rasogolla is a soft, succulent ball of chhana soaked, stored, and served in sugar syrup. Snow-white in color, it possesses a spongy, chewy body, and smooth texture. The Bureau of Indian Standards has laid down the standards for rasogolla, as shown in (Table 27.3). Its variations include rasmalai, and rajbhog. Rasogolla is prepared by

TABLE 27.3 Standard Specifications for Rasogolla.

Characteristics	
Moisture	45–55%
Milk fat	5%
Sucrose	45%
Protein	5%
Requirements for Syrup	
Acidity of syrup (mL of N/10 NaOH required to neutralize 100 mL of the syrup) (maximum)	6.0
Concentration of syrup (maximum)	55° Brix
Bacterial count, per gram (maximum)	500
Coliform count, per gram	Nil

texturization of milk protein under controlled cooking conditions in boiling syrup. This gives rasogolla an interwoven fiber-like structure. The controlled cooking in sugar syrup imparts spongy characteristics to rasogolla balls and improves their sugar-holding capacity. The unique spongy characteristic of rasogolla is typical for this dairy confection.

Rasogolla is prepared from soft, freshly made chhana, which is first manually kneaded to a smooth paste (Fig. 27.3). The paste is then portioned and rolled between palms to form balls of about 15 mm in diameter and around 10 g in weight. Each ball should have a smooth surface with no cracks. In some cases, chhana needs to be modified by mixing it with 6% arrowroot, 2% semolina, and 0.6% baking powder (of the chhana weight), and subsequently kneaded manually to a smooth paste. One kilogram of chhana typically yields 90–100 rasogollas.

27.2.2.1 Cooking Medium. For preparation of the cooking medium, three parts sugar are mixed with one part whey and two parts water. The pH is adjusted to about 6.8 with the addition of calcium hydroxide. The solution is boiled, and the scum that forms on the top is scooped off before it is subjected to further cooking. rasogolla balls are dipped in the cooking medium. The heating of the syrup is regulated to preserve the round form from cracking. The balls are cooked for about 15 min. During cooking, a small amount of water and whey solution is continuously added. This makes up for the loss of water due to evaporation. The ratio of whey and water is adjusted to maintain the pH of the medium at around 6.8 during cooking. After cooking, the balls are transferred to a container containing water at 30–35°C for stabilization of their texture and improvement of color. About 10% of the cooking solution is replaced by fresh sugar solution for the next batch of rasogollas.

27.2.2.2 Sugar Syrup. After 5–10 min of texture stabilization in water, the balls are transferred to sugar syrup. The desired sugar syrup concentration in the final product is 45–50%. This is achieved by dipping the texture-stabilized balls first in 50–60% sugar syrup for 1–2 h, followed by a second dipping in 40–50% sugar syrup. Finally, the

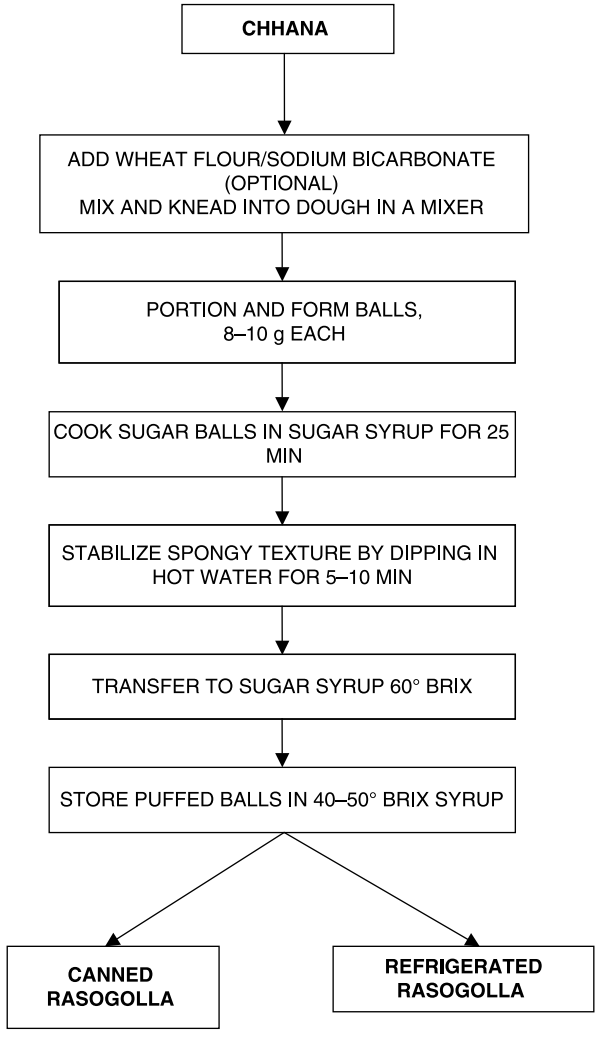

Figure 27.3 *Steps in the manufacture of rasogolla from chhana.*

product acquires the desired sugar concentration after equilibrium is reached between the sugar syrup inside the balls and outside them. To exercise better control over the sugar syrup concentration in the finished product, dipping of the texturized balls may be done in three stages. In the first stage, dipping is in a 60% sugar level, followed by second-stage dipping at 40% concentration, and final dipping at 50% concentration. Rasogollas are stored at or below 10°C to obtain a more precise sugar concentration of 45–50%.

27.2.2.3 Yield. The yield of finished rasagolla (drained) is 254 g/100 g chhana. Typically, about 90–100 rasagollas of diameter 30 mm can be made from 1 kg of chhana.

27.2.2.4 Packaging. Rasogollas are usually packaged in lacquered tin cans of 1 kg capacity, containing approximately 14 pieces. The empty containers are first sterilized

with hot air inside a closed chamber. Rasogolla balls with hot sugar syrup and permissible preservatives are filled in containers, followed by seaming, which tightly seals the lid. Sodium metabisulfite is used to maintain the white color of canned rasogollas. Containers are kept in a tub containing chilled water to create a partial vacuum by quick condensation of vapors inside the container. The shelf-life of rasogollas in tin containers is around six months.

27.2.2.5 Rasmalai. Rasmalai consists of flattened chhana patties floating in concentrated sweet milk. It has a delicate chewy/spongy texture. Chhana is kneaded into a smooth dough along with 1–4% wheat flour. The dough is portioned and rolled into balls having a smooth texture without cracks. The round balls are then flattened into patties and are processed like rasogolla. They are subsequently stored in sweetened (5–6% sugar) milk condensed to one-fourth of its volume. In many cases, milk is blended with cream.

27.2.2.6 Rajbhog. Rajbhog is also a variety of rasogolla, but is larger in size than rasogolla (about 50–60 mm in diameter). Compared to rasogolla, rajbhog balls are almost twice as large. Rajbhog is blended with saffron, which imparts an attractive golden yellow shade to it. Chhana is kneaded into a uniform dough, mixed with a small amount of saffron, and portioned and shaped into balls with hands. While shaping, a raisin or a nut is placed at the center of the ball. The balls are cooked in a 50% solution of boiling sugar syrup. Cooking is continued until a desirable body and texture is achieved. Thereafter, the balls are removed from the syrup and may be wrapped in silver foil.

27.2.3 Sandesh

Sandesh is known for its palatability, aroma, and bar-like shape. It has a firm body and smooth texture. It is a rich source of milk proteins, fat, sucrose, and fat-soluble vitamins. Three distinct varieties of sandesh are popular: soft grade (*naram-pak*), hard grade (*kara-pak*), and raw grade (*kachagolla*). The most common variety is the soft-grade sandesh. It has a soft body and smooth texture with fine grains uniformly distributed. It contains a relatively smaller amount of sugar and has higher moisture content than the hard-grade variety. *Kara-pak* has a firm body and dry appearance. Kachagolla is known for its raw chhana-like flavor, moist appearance, soft body, and coarse, grainy texture.

The production process for soft-grade sandesh is described in Figure 27.4. Chhana (30–45%) and sugar are mixed and kneaded together and heated in a shallow vessel after addition of color and flavor. The chemical profiles of commercial samples of three types of sandesh are listed in Table 27.4.

Some attempts have been made to apply extrusion technology to the manufacture of sandesh. A vented extruder with vent ports in the barrel for the volatiles and moisture to escape during the continuous process is feasible. A screw-type kneader can be used for mixing chhana and sugar to achieve the homogeneity of mixture critical for sandesh manufacture. Sandesh produced by this continuous production method has been shown to be superior in quality in comparison to the market product (Agarwal 1996). The shelf-life of sandesh produced in this process and packed in polyethylene bags was 16 days at $10 \pm 5^{\circ}C$.

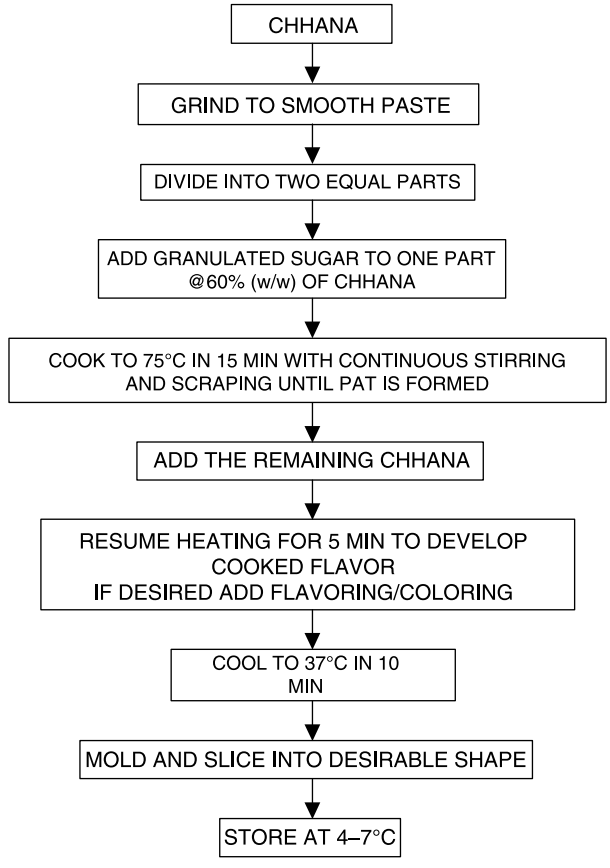

Figure 27.4 *Production process for soft–grade sandesh.*

TABLE 27.4 Physico-Chemical Attributes of Market Samples of Sandesh (Average Values Based On Market Survey).

Attribute	Soft Grade (30 samples)	Hard Grade (15 samples)	Kachagolla (15 samples)
Moisture (%)	24.1	13.4	33.9
Fat (%)	18.7	20.5	15.5
Protein (%)	16.1	16.7	12.8
Sucrose (%)	38.6	46.4	35.8
Ash (%)	1.7	1.7	1.4
Titratable acidity (% LA)*	0.88	0.82	0.7
Water-soluble acidity (% LA)*	0.35	0.22	0.19
FFA (% OA)**	0.41	0.34	0.46
Free fat (% of total fat)	68.6	80.8	56.9
Hardness (kg)	4.7	–	–
Cohesiveness	0.21	–	–
Springiness	0.30	–	–
Gumminess	1.0	–	–

*Expressed as % lactic acid.
**Expressed as % oleic acid.

27.3 PANEER

Paneer is also an acid-coagulated product of hot milk. It resembles chhana in its manufacturing procedures, but differs in that it is lightly pressed into blocks. It resembles tofu in appearance and texture, but possesses a distinct flavor of its own. Paneer is an integral part of Indian culinary preparations. Paneer offers outstanding nonmelt functionality because of its ability to withstand cooking and frying temperatures. This unique characteristic permits its use in Indian cuisine. Paneer lends itself to the preparation of fried cheese snacks. The Pure Foods Act of India requires paneer to have not more than 70% moisture and not less than 50% fat on a dry matter basis. Skim milk paneer should contain not more than 70% moisture and not more than 13% fat on a dry matter basis.

27.3.1 Manufacturing Procedure

Paneer is typically made from buffalo milk in South Asia. It is also manufactured on an industrial scale in the UK, Holland, Canada, and the United States, using cow's milk. Paneer is obtained through heat/acid coagulation of casein component of standardized milk, entrapping through various interactions almost all the fat, part of the denatured whey proteins, and colloidal salts as well as a part of the soluble milk solids (in proportion to the moisture content retained). The typical chemical composition of paneer is: 53–55% moisture, 23–26% fat, 17–18% protein, 2.0–2.5% lactose, and 1.5–2.0% minerals. Paneer is marble white in appearance with a slight spongy-body and close-knit texture. It possesses a mild sweetish-acidic nutty flavor.

The paneer production process is illustrated in Figure 27.2, and shows that blocks of paneer are obtained by pressing chhana in molds/hoops. Various steps for paneer production are discussed in the following.

27.3.1.1 Milk Standardization. The milk used for making paneer should be standardized to a fat/solids-not-fat (SNF) ratio of 1 : 1.65 in order to achieve a minimum of 50% fat in dry matter (FDM) in the finished product. To achieve this standard, the starting milk must have a minimum of 5.8% milk fat. In India, buffalo milk or blended milk is the preferred milk because it provides higher fat (6%) and SNF (9.5%) contents as compared to cow's milk. In the West, the quality of paneer made from cow's milk is quite acceptable. Cow's milk is normally blended with 0.1% calcium chloride to ensure a good yield. High-quality paneer with 42% fat (FDM) can be obtained from milk composed of 3.5% fat and 8.5% SNF. It has a soft meaty texture and a pleasant, creamy flavor. Low-fat paneer is also available, which contains fat in the range of 24% FDM. Although skim-milk paneer containing 13% FDM is feasible, the product has a chewy, rubbery and hard body.

27.3.1.2 Heat Treatment. In a typical plant, milk standardized to 5.8–6.0% fat (9.5% SNF) is heated in a plate heat exchanger from 4°C to 85–90°C and pumped into a water/steam jacketed cheese vat and allowed to cool to 70–75°C in about 10 min prior to acid blending.

27.3.1.3 Curd Formation. Industrial processes utilize citric acid as the coagulant of choice. Glacial acetic acid is also used by some manufacturers. The type and concentration of the acid and the mode of delivery and blending into the hot milk directly influence product yield and moisture retention.

Citric acid is generally used in a 1–2% solution. The preferred concentration is 1% citric acid in the coagulant, which is warmed to 70°C. Sufficient acid is gently but quickly blended with the milk (within 1 min) to drop the pH to 5.3–5.4. Normally it requires about 1.8–2.0 g of citric acid to coagulate 1 kg of milk. At this point, clear greenish-yellow whey separates out, allowing the curd to sink to the bottom.

Coagulation temperature influences the moisture content of paneer. An increase in temperature from 60 to 90°C decreases moisture in the paneer from 59 to 49%. Paneer obtained at a coagulation temperature of 70°C has the best organoleptic quality as well as the most desirable frying quality in terms of shape retention, softness, and integrity maintenance. Variation in pH of coagulation has a profound effect on processing and quality parameters. For example, the moisture content of paneer increases from 50 to 59% and the yield increases from 21 to 25% when the coagulation pH is increased from 5.1 to 5.4. Sensory quality is judged as best at pH 5.30–5.35, the recommended pH for coagulation.

27.3.1.4 Whey Drainage. The coagulated milk is allowed to separate into curd and clear whey. After 10 min, practically all the curd chunks sink to the bottom. At this point, a strainer is fitted into the outlet of the cheese vat. The whey drainage valve is then opened, and the whey flows into a surge tank partitioned by a strainer to retain any curd fines escaping from the cheese vat. Hot whey accumulates in the second section of the surge tank from where it is pumped out, cooled to 4°C, and stored in a whey tank for further processing or disposal.

27.3.1.5 Hooping. Normally, the curd is transferred to hoops lined with cheesecloth. The hoops have perforations on all sides to facilitate whey expulsion. Hoops may vary in size from 2 to 10 kg in capacity. For culinary purposes, unsalted paneer is traditionally used. Prior to placing the lid on the hoop and applying pressure, extra curd is added to facilitate block formation of paneer.

27.3.1.6 Pressing. The hoops are pressed using a hydraulic press to exert a low pressure (6.9–13.8 kPa) onto the curd. The pressing time is generally around 10–15 min after which the wrapped blocks of paneer are ejected from the hoop for quick cooling. Moisture content, shear strength, and porosity of paneer are a function of the pressing conditions. For optimum sensory quality, pressing paneer blocks at 9.8 kPa is recommended.

27.3.1.7 Cooling. The cooling of the wrapped blocks is effected by immersion in cold water (4°C) containing 3–5 ppm chlorine for 1–2 h. During dipping, paneer blocks absorb enough water to develop the typical body and texture. Another objective of dipping in cold water is to cool the blocks to less than 20°C, which facilitates handling during cutting and wrapping for retail sale. The blocks are then mechanically cut into convenient retail sizes (200 g or 500 g), and packaged into pouches made up of appropriate packaging film to maintain paneer safety and integrity during distribution and retailing.

27.3.1.8 Packaging. Various packaging materials used by manufacturers of paneer include coextruded laminates of moisture-barrier and oxygen-barrier films, polyethylene sachets, or heat-induced-shrink film. Paneer must be protected from drying out and exposure to oxygen (air) during storage. The product packaged in moisture-barrier film pouches, followed by vacuum application and heat-sealing, provides a shelf-life of over

6 months of refrigerated storage. Quick-freezing extends the shelf-life. For distribution over long distances and storage for extended periods of time, it is advantageous to freeze paneer packages.

27.3.1.9 Yield. The yield of paneer is dependent on the fat and SNF content of the starting milk, as well as on the moisture, fat, and protein retained in the paneer. A yield of 21–23 kg of paneer containing 51–54% moisture per 100 kg of buffalo milk has been achieved. This yield corresponds to a 63–67% milk solids recovery in the paneer. With cow's milk, paneer yield is around 17–18%. By the application of membrane technology (ultrafiltration), the paneer process has been improved.

27.3.1.10 Defects. Many defects in the flavor and texture quality of paneer can be traced back to the microbiological and organoleptic attributes of the starting milk. Table 27.5 shows some commonly found defects in paneer and also methods to prevent them.

27.3.1.11 Physico-Chemical Changes During Paneer Manufacture. The coagulation process in paneer manufacture occurs because of the chemical and physical changes in casein brought about by combined heat and acid treatment. This treatment leads to formation of large structural aggregates of casein from the normal colloidal dispersion of discrete casein micelles in which milk fat and coagulated serum proteins are entrapped together with whey. During formation of coagulum, major changes include the progressive removal of tricalcium phosphate from the surface of the casein and its conversion into monocalcium phosphate. Further, calcium is progressively removed from calcium hydrogen caseinate to form the soluble calcium salt and free casein.

TABLE 27.5 Defects Observed in Paneer and Measures for Their Prevention.

Defects	Preventive Measures
	Flavor
Sour	Use fresh milk having no developed acidity. Follow recommended coagulating acid concentration, temperature, and volume parameters
Oxidized/rancid	Store paneer at 4°C to avoid hydrolysis/oxidation of milk fat
Stale/foreign	Store paneer at 4°C to control microbial growth
	Body and Texture
Coarse texture	Use fresh milk. Standardize fat : SNF ratio to 1 : 1.65 Standardize coagulation temperature (70°C and pH 5.3)
Hard body	Optimize fat in milk (5–6%) Standardize coagulation, heating, and cooling conditions during manufacture
	Appearance
Dry surface	Optimize fat content of milk
Surface hardening	Avoid surface drying during manufacture Package in better moisture–barrier packaging material
Unclean	Insist on sanitary practices and use good-quality milk
Brown coloration/spots	Avoid overhead surfaces of equipment

27.3.1.12 Microstructure of Paneer. The manufacturing process and milk components, such as casein micelles, whey proteins, fat globules, lactose, and minerals, determine the texture and microstructure of paneer. Microstructure also influences some physical properties of the product such as firmness, elasticity, susceptibility to syneresis, and mouth feel. For studying the microstructure of dairy products, scanning electron microscopy (SEM) has proved to be a very useful tool, especially in tandem with other electron microscope techniques.

In paneer, the building units, namely casein micelles, are several times larger than in the starting milk. The casein micelle swells from 100 to 300 nm as a result of heat treatment to the milk. Gels prepared by coagulating the hot milk with citric acid possess a coarser structure than heat-induced milk gels. The most significant character of the gels obtained by coagulating milk heated to 90°C at pH 5.5 is the formation of a unique structure form the casein particles. Development of such a core-and-lining structure depends on the temperature at which the milk is coagulated and the final pH value.

Cow-milk paneer resembles American cottage cheese in microstructure, with a uniform density of small protein particles. In contrast, buffalo-milk paneer has more densely packed and fused protein particles. Intact fat globules with casein particles attached to the fat globule membranes are frequently seen in unfried fresh paneer. Frying in vegetable oil has a massive impact on the paneer structure. The SEM shows that compaction severely suppresses or even destroys the granularity of the protein matrix observed in unfried fresh paneer. Apart from the solid compaction of core-and-lining structure resulting from frying, the presence of deviated core-and-lining structures can also be seen at higher magnifications. Cooking in salt water restores both the granular structure and the core-and-lining structure of the protein particles.

27.4 LATIN AMERICAN WHITE CHEESES

Latin American white (LAW) or Hispanic cheeses (Queso Blanco) are found throughout Mexico, Central and South America, and the Caribbean Islands. They are known by various names (Table 27.6). These cheeses are made from whole milk, skim milk, cream, or their mixture. The use of coagulating agents varies with the type of cheese. The production process involves rennet coagulation of warm milk or curdling of hot milk with lime/lemon juice, fruit juice, or vinegar. Directly acidified cheeses include *queso del pais*, *queso de la tierra*, *queso de cincho*, and *queso sierra*. Cheeses obtained by rennet coagulation include *queso fresca*, *queso de prensa*, *queso de puna*, *queso de hoja*, *queso de matera*, and *queso pasteurizado*. They are all highly salted (salt level 2–4%) to improve their shelf-life. Generally, Hispanic cheeses are white, creamy in taste, highly salted, and acidic in flavor. They possess the body and texture of young, high-moisture cheddar and can be sliced for sandwich use.

By flavoring the LAW cheese curd with onion, garlic, caraway seeds, or hot pepper spices, an array of varieties of flavored Hispanic cheeses can be marketed. These can be used as a snack in salads, as cooking cheese in casserole dishes, grated for use in pizza and other foods, or included as an ingredient in the manufacture of processed cheese. For producing process cheese with LAW cheese (renneted), the formulation involves 2.05 kg of ground LAW cheese, 113.5 g butter, 178.8 g water, and 56.8 g disodium phosphate emulsifier. These ingredients are cooked in a steam-jacketed kettle to 70°C for 4 min. After holding for 1 min, the mixture is cooled in polyethylene-lined

TABLE 27.6 Origin and Nomenclature of Some Latin American White Cheeses.

Name	Country of Origin
Fresh Skim-Milk Cheeses	
Queso de Puna	Puerto Rico
Queso Fresco	El Salvador and Venezuela
Queso Llanero	Venezuela
Queso de Maracay	Venezuela
Queso Descremado	Costa Rica and Queso Huloso
Whole Milk/Part-Skim-Milk Cheeses	
Panela	Mexico
Queso de Prensa	El Salvador and Venezuela
Queso del Pais	Puerto Rico
Queso de la Tierra	Puerto Rico
Queso de Estera	Colombia
Queso de Crema	Panama
Grating	
Queso de Bagaces	Costa Rica

boxes. The inclusion of aged cheddar cheese for flavor gives a very desirable processed cheese. Such LAW cheese is largely consumed fresh, but can be fried with or without butter to prepare nutritious snacks of excellent eating quality. The pressed cheese is hard and crumbly, with a slightly open texture. To improve flavor, starter cultures or lipases may be incorporated prior to salting. In the treated cheese the pH drops during storage. Also, several volatile flavor compounds are generated. Consequently, the treatment leads to the development of a cheddar-like cheese flavor.

27.4.1 Manufacturing Procedure

LAW cheeses are made on farms as well as in industrial plants. The farm product is soft or semisoft cheese and is called *queso de matera* in Venezuela. The industrial product is relatively soft and is called *queso pasteurizado*. The farm cheese is made from raw milk and is highly salted (8–25% salt on wet basis). Consequently, it acquires a drier body than the industrial product. Transportation time from farm to consumer varies from days to weeks and may result in some ripening changes. In the farm cheese production, direct acidification and rennet coagulation are interchangeably used for curd formation. *Queso enchilado* is based on renneted cheese curd, which, after pressing as a 12-kg block and holding for 10 days, is immersed in chili sauce to give it a spicy, hot taste. *Queso oaxaca* is a soft cheese resembling mozzarella. Pasteurized milk, starter, and rennet are its basic materials. The curd is cooked at 39°C at a pH of 5.2. Dry salting to a level of 4–5% salt follows stretching of the curd by hand in hot water. While hot, the curd may be molded into various forms, including braided shapes; it is then cooled and packaged in plastic pouches. The methodology for LAW cheese manufacture differs in the many manufacturing countries. Variations in procedures on the farm and in industrial plants are common. A typical manufacturing procedure for LAW cheeses is shown in

Figure 27.2. The procedure is similar to paneer manufacture, except the addition of salt to the LAW cheese curd. It also differs from paneer in that the blocks of pressed cheese are not immersed in cold water.

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28

Cream Cheese as an Acidified Protein-Stabilized Emulsion Gel

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28.1 Introduction 652
28.2 Building Structure: Gelled O/W Emulsions or the Science Behind Cream Cheese 652
28.2.1 Ingredients 652
28.2.2 The Emulsion as a Composite Material 655
28.2.2.2 Emulsion Structuring by Controlling the Continuous Phase 661
28.3 Cream Cheese Processing 663
28.3.1 Emulsification 663
28.3.2 Acidification 664
28.3.3 Separation 664
28.3.4 Filling 665
28.4 Cream Cheese Properties 665
28.4.1 Spreadability 665
28.4.2 Melting in the Mouth 666
28.4.3 Syneresis 666
28.5 Cream Cheese Properties During Transport and Storage 667
28.6 Summary 667
References 667

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28.1 INTRODUCTION

Throughout history, milk products have been important elements of human nutrition in many parts of the world. Unfortunately, fresh dairy products like milk spoil relatively easily because microorganisms thrive well on them. Several obvious routes can be used to improve the keepability of these products:

- Acidification through fermentation (e.g., in yoghurt),
- Decreasing the water activity (e.g., in hard cheese),
- Pasteurization or sterilization, and
- Appropriate packing of the finished product (Shephard 2000).

These preservation techniques have been identified in many different cultures all over the world, and their implementation has resulted in a wide range of similar but slightly different local dairy products. In this chapter we will focus on acidified cheeses. Well-known examples of such products are cream cheese, cottage cheese, fromage frais, and full-fat quark (Guinee and others 1993).

The purpose of this chapter is to provide an overview on structure formation in cream-cheese-type products, which can be grouped under acidified milk protein-stabilized emulsion gels, with an emphasis on the emulsion aspects of such systems. As such, this chapter is complementary to the excellent review published earlier by Guinee and others (1993), which emphasized protein structuring in these products.

To produce a protein-stabilized emulsion gel, a fat phase has to be emulsified, for example, via homogenization, in a continuous phase. Furthermore, the emulsion has to be structured to achieve the required textural properties. Combined gelling of the continuous phase and crystallization of the dispersed phase achieve structuring of protein-stabilized emulsion gels. The firm, crystallized fat droplets will reinforce the emulsion gel by adhering to the continuous phase by means of interfacial protein.

After a brief introduction on milk proteins and emulsions, structure formation (protein denaturation, heat- and acid-induced gelling, fat crystallization, [partial] coalescence) will be reviewed in the context of cream-cheese processing (separation, heating, emulsification, acidification). The role of both dispersed and continuous phases on properties will be discussed, with emphasis on those aspects that are important in relation to cream-cheese texture (firmness, syneresis, spreadability, emulsion stability during temperature cycling) and taste.

28.2 BUILDING STRUCTURE: GELLED O/W EMULSIONS OR THE SCIENCE BEHIND CREAM CHEESE

28.2.1 Ingredients

Full-fat milk consists of water, milk protein, butterfat, lactose, colloidal calcium phosphate, and some minor components (Walstra and Jenness 1984). For industrial application, full-fat milk is often fractionated in protein-rich and fat-rich preparations, each of which can serve as the basis for further products.

Proteins in milk can be classified mainly in two groups: casein and whey protein. Casein is present in milk in the form of small protein aggregates, called casein micelles,

typically of 50–300 nm diameter. Each micelle consists of a mixture of α-, β- and κ-caseins, all proteins with relatively little secondary or tertiary structure. Various models exist for the structure of these micelles, but currently the most explanatory appears to be the version where α-casein forms the structural backbone of the micelle (linked via either direct protein–protein interactions or Ca-bridged interactions), supplemented by β-casein and colloidal calcium phosphate, and where most of the κ-casein can be found at the surface of the micelle (Horne 1998; Mellema and others 1999). The protein κ-casein is a block-polymer-like protein consisting of a relatively hydrophobic part and a relatively hydrophilic part. The hydrophobic part anchors itself in the micelle, whereas the hydrophilic part (known as the casein macropeptide, CMP, or as the glycomacropeptide, GMP, if glycosylated) acts more or less as a salted polyelectrolyte polymer brush (de Kruif and Zhulina 1996; Tuinier and de Kruif 2002). This brush stabilizes the micelles against aggregation because the charged carboxylic acid groups in the hydrophilic part of the κ-casein repel each other weakly. The stabilization is retained as long as the κ-casein remains negatively charged.

For unheated milk, the globular whey protein is in its native form and does not contribute to the aggregation behavior of the milk. Whey protein is a mixture of β-lactoglobulin (∼65%), α-lactalbumin (∼25%), and serum albumin (∼8%), which are soluble in their native forms, independent of pH. Prolonged heat treatment at sufficiently high temperatures and long duration will denature (i.e., partly unfold) the whey protein, triggering hydrophobic interactions with other proteins and interfaces, and the formation of disulfide links between whey proteins and between whey proteins and micelles. Depending on the conditions (e.g., pH) under which this heat treatment is applied, the whey protein may form separate aggregates or cover the casein micelles (Creamer and others 1978; Heertje and Pâques 1995). Such heat treatments change the pH below which milk gel formation occurs (Vasbinder and others 2001). A number of physical properties of milk proteins are summarized in Table 28.1.

Butterfat is composed of a mixture of triacylglycerols (TAGs, or triglycerides) referred to as fats and oils. The liquid phase is called the olein fraction, and does not contain any crystallized TAGs at ambient temperature. The crystalline fraction is called the stearin fraction. The amount of crystalline fat ("solid fat content" or SFC) as a function of

TABLE 28.1 Some Physical Properties of Individual Milk Proteins: Concentration by Weight in Milk Protein, Isoelectric Point *p*I, Molecular Weight M_w, and Calcium Binding Activity.

Concentration	(%)	*p*I	M_w	Calcium Binding
		Casein		
α_{S1}-casein	31	4.2–4.6	23,614	++
α_{S2}-casein	8		25,230	++
β-casein	28	4.6–5.1	23,983	+
κ-casein	10	5.3–5.8	19,023	−
		Whey Protein		
β-lactoglobulin	9.8	5.1–5.3	18,283	−
α-lactalbumin	3.7	4.2–4.5	14,176	+
BSA	1.2	4.7–5.1	66,267	−
IgG, IgM, IgA...				

BSA, borine serum albumin; Ig, Immunoglobulin.

temperature is routinely measured using NMR techniques, and is often referred to as the n-line of the fat (Gribnau 1992).

TAG crystals occur in a number of different polymorphic forms α, β′, β, that is crystal structures with different states of molecular packing (Bruijne and Bot 1999; Bot and others 2003a). The preferred polymorphic form of fat depends on the chemical composition of an individual TAG, the mixture of TAGs in the liquid oil, and the crystallization conditions. Transformations between polymorphs depend, amongst others things, on the mobility of the TAGs in the crystal and liquid phases, and the ease with which molecules fit within the new crystal lattice. In commercial fat sources like butterfat, the presence of large numbers of different species of TAGs makes crystallization behavior rather complex (Coultate 1996; Campbell and others 2002). In practice, butterfat is found to crystallize usually in the β′ form, as illustrated in Figure 28.1. Amongst the minor ingredients, the carbohydrate lactose is the most important one. Lactose contributes to the sweetness of milk. Lactose is the main nutrient for lactic acid bacteria during fermentation. Typically, milk or milk powder contains ~50% lactose on dry matter, but other milk protein preparations may contain much more (e.g., sweet whey powder) or much less (whey protein isolate) lactose. Although the solubility of lactose is sufficiently high to prevent crystallization in cream-cheese-type products under normal conditions, it can manifest itself occasionally in the form of a certain degree of grittiness of the final product.

Calcium, an important nutrient in milk, is mainly present in the micelle in the form of colloidal calcium phosphate (CCP). Calcium promotes aggregation of α-casein mainly. The concentration of CCP is an important factor determining the stability of the micelles and the buffer capacity of a protein powder.

Phospholipids are surface-active materials, and as such can be found in fat-rich fractions like cream or even buttermilk powder because these preparations contain most of the membrane material. Phospholipids compete with the protein at the interface, and may decrease the interaction between droplets and matrix in the acidified emulsion (see Section 28.2.2).

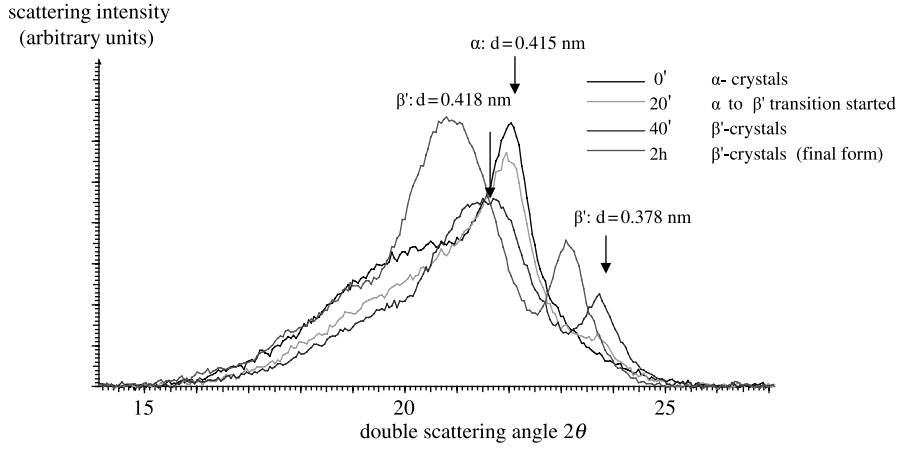

Figure 28.1 *X-ray diffractogram of a 30% butterfat model emulsion stabilized by 4% whey protein. The position of the diffraction peaks reveal the initial formation of α polymorphs, followed by the formation of β′-polymorphs over longer time scales. The vertical scale for each individual diffractogram was chosen to allow for convenient plotting (unpublished data from S. Kiokias and E.C. Roijers, Unilever Research and Development).*

Summarizing, full-fat milk can be considered the source of the two main ingredients of cream cheese: butterfat and protein. In practice, full-fat milk is not separated in butterfat and protein but centrifuged to form a fat-rich phase, cream, and a protein-rich phase, (skimmed) milk. The protein-rich phase can be processed/fractionated further to form other protein powders, such as whey protein concentrates and isolates. By mixing the fat-rich and protein-rich phases together again in different proportions, it is possible to manufacture dairy products that have completely different relative contents of fat and protein than the original full fat milk.

28.2.2 The Emulsion as a Composite Material

Cream cheese can be characterized as an (acid-protein-stabilized) o/w emulsion microstructure, in which attraction between the proteins at the surface of the butterfat droplets causes the droplets to aggregate into clusters (Fig. 28.2). Surplus protein tends to aggregate onto these clusters as well, leading to a protein network around the protein aggregates. Intense flow (shear, elongation, turbulence), as for example applied during homogenization, may lead to a (partial) collapse of this protein network onto the droplet aggregates.

As the droplets in the fat–protein aggregates usually remain present as individual droplets, the overall structure of the product can be characterized best as a composite (Fig. 28.3): a protein matrix filled with fat droplets. The dispersed phase acts as filler particles, the continuous phase as matrix. The properties of the filler phase affect the final product properties as a result of adhesion between droplet and filler phase. These effects become more important at higher filler fractions. When the interaction between filler and matrix becomes less, for example, when protein is replaced at the droplet interface by other emulsifiers, the contribution of the filler phase to the hardness becomes less

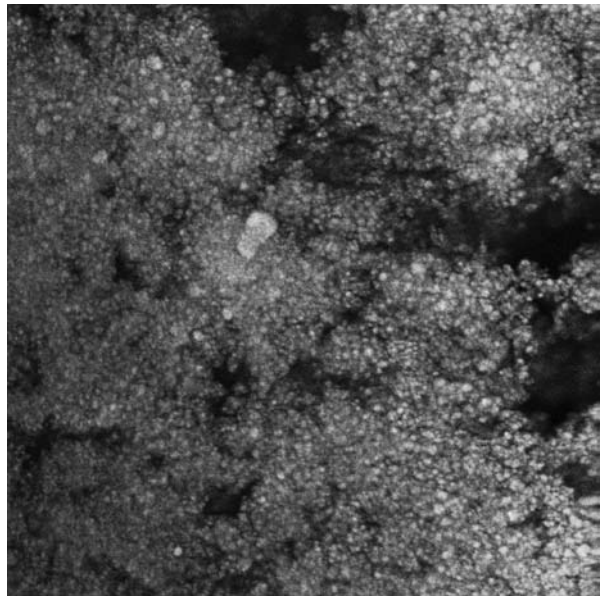

Figure 28.2 *Confocal scanning light microscopy image of the aggregated fat droplet microstructure in a commercial cream cheese. Image width 260* μm *(Image by H. van Aalst, URDV.).*

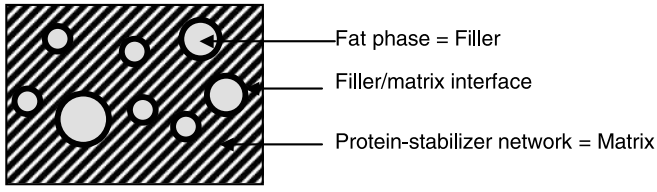

Figure 28.3 *Schematic representation of the microstructure of a protein-stabilized emulsion gel as a composite material.*

important (van Vliet and Dentener-Kikkert 1982; Reiffers-Magnani and others 1999). Effects of volume fraction, droplet size, and rigidity of the dispersed phase on composite gel strength and interaction with the continuous matrix have been investigated extensively (Jost and others 1986, 1989; Aguilera and Kessler 1988, 1989; van Vliet 1988; Langley and Green 1989; Aguilera and Kinsella 1991; Xiong and others 1991; Yost and Kinsella 1992, 1993; Aguilera and others 1993a; McClements and others 1993b; Wijmans and Dickinson 1998; Cho and others 1999; Aguilera 2000; Anton and others 2001). The spatial distribution of the dispersed phase can be expected to be important also.

Figure 28.4 shows some general dependencies of physical parameters on filler characteristics in model emulsion gels. Composite firmness tends to increase with filler concentration, and the effect is more pronounced if the filler particles are firm (Fig. 28.4*a*) (Xiong and others 1991). There are two contributions to this effect. In part, there is a direct effect of the increased hardness of the emulsion droplets (liquid oil being "soft" and crystalline fat being "hard") on the firmness of the composite. This is illustrated in Figure 28.5 by the effect of temperature on the firmness of a commercial fresh cheese, reflecting the melting of the fat phase. In part, concentration of the continuous phase plays a role (because fat occupies a larger volume and the protein in the continuous phase is more concentrated and hence forms a firmer gel; Aguilera and Kessler 1989; Aguilera and others 1993b; Dickinson and Hong 1995; Jost and others 1986). This represents an indirect effect of the fat droplets on composite strength, as it is linked to the strength of the continuous phase. Furthermore, there is an effect of the gelling-inducing processing steps, like heating (Reiffers-Magnani and others 1999) and acidification, on the composite properties of whey protein-stabilized o/w emulsions.

At a constant fat concentration, droplet size influences the strength of the composite (Fig. 28.4*b*) (Xiong and others 1991), a higher composite strength being observed with smaller droplets, independent of their physical properties. This is probably caused by the larger number of interactions between proteins at the interface and those in the continuous phase upon increasing the surface area, thus strengthening the adhesion and resulting in gel reinforcement (Ross-Murphy and Todd 1983; Langley and others 1988; van Vliet, 1988; Aguilera and Kessler 1989; Jost and others 1989; Xiong and Kinsella 1991; Yost and Kinsella 1993). This statement is only valid if the emulsifier adsorbed at the oil/water interface interacts with the continuous phase (for example, in the case of dairy gels containing milk protein as emulsifier), and if the droplets are sufficiently small to behave like (slightly deformable) hard spheres under small deformations (like the $\sim$1 μm droplets in cream cheeses). Low-molecular-weight emulsifiers tend to interact weakly with the protein in the gel network, leading to softer emulsion gels, and for this reason protein is more effective as the main emulsifier in cream cheese to achieve firmness in the final product.

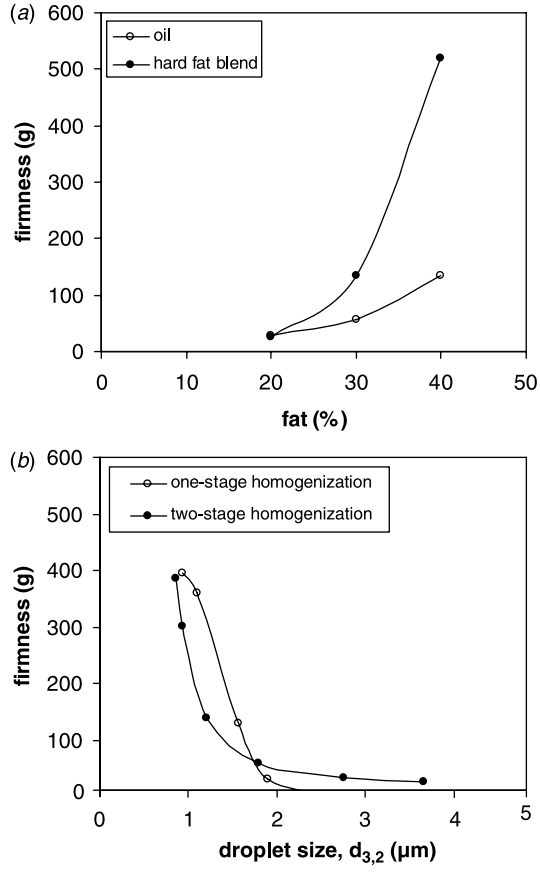

Figure 28.4 *Influence of dispersed phase of a composite, an acidified model emulsion, on firmness at 5°C as measured in a penetration test. (a) Effect of filler volume for a soft filler (oil) and hard filler (hardened vegetable fat) at roughly constant droplet size for an emulsion with 3% whey protein. (b) Effect of droplet size (i.e., different homogenization pressures) for 30% fat + 3% whey protein emulsions containing hardened vegetable fat. Two-stage homogenization involves a low-pressure second homogenization stage that tends to decluster the emulsion droplets (giving lower firmness at high first-stage homogenization pressure), but decreases the width of the droplet size distribution (giving higher firmness at low first-stage homogenization pressure).*

28.2.2.1 Emulsion Structuring by Controlling the Filler Phase

Milk Proteins as Emulsifiers. A (macro)emulsion is not a thermodynamically stable system and therefore does not form spontaneously. Creation of an oil-in-water emulsion involves dispersing oil in the form of droplets in the aqueous phase, usually under high shear conditions in a high-pressure homogenizer. The high shear stresses during this process lead to turbulence, breaking of the oil phase and formation of droplets. The droplet diameter obtained in such a process depends on the mechanical energy input per volume dispersed oil and the interfacial tension γ between the dispersed and the continuous phases (Walstra and Jenness 1984). The interfacial tension is reduced by the addition of emulsifiers, such as proteins or low-molecular-weight emulsifiers (e.g., monoglycerides, phospholipids), which contain both apolar regions that anchor to the fat phase and polar

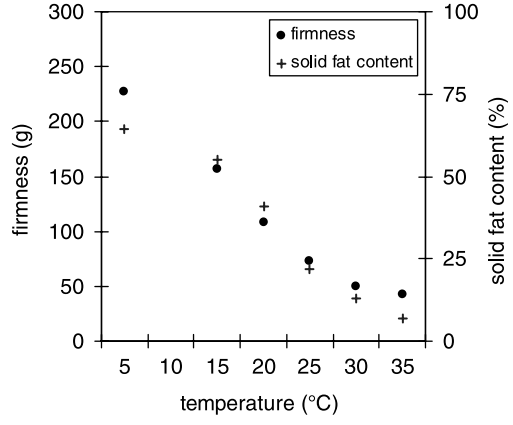

Figure 28.5 *Firmness measured by a penetration test for a commercial cream cheese as a function of temperature. Also indicated is the melting curve of butterfat (data by M.H. Schnitker, URDV).*

regions that orient towards the aqueous phase. Details on milk protein structure and behavior at the oil–water interface are given elsewhere (Leman and Kinsella 1989; Dickinson 2001).

Emulsifier functionality has two important aspects: the creation of a finely dispersed phase and the long-term stability of the emulsion. The first aspect is covered by using emulsifiers that can rapidly adsorb at the interface, and thus reduce the interfacial tension of the newly created droplet interface during stretching of the droplet in the homogenizer and subsequent break-up. This needs to be a rapid process, because it needs to be completed within the typical μs pass-through timescale for the homogenizer valve. A slower adsorption process would allow significant recoalescence of the dispersed droplets. As small molecules diffuse faster to the oil–water interface, the effectiveness of the emulsifier for making small droplets tends to reduce with increasing molecular weight (making low-molecular-weight emulsifiers more effective than proteins, for example).

Comparing different protein sources, this implies that single protein molecules like caseinate and native whey protein are more effective in emulsification than bulky casein micelles (Dickinson 1992, 1993; Euston and Hirst 2000). Droplet size was found to increase when emulsions were prepared from a range of commercial milk protein powders, micellar casein (MC), skim milk powder (SMP), and whey protein concentrate (WPC), according to WPC $<$ SMP $<$ MC (Euston and Hirst 2000). Conditions that promote protein aggregation, such as denaturation and/or acidification, reduce the effectiveness of a protein in the emulsification process.

The second aspect, however, is the long-term stability of the emulsion against partial coalescence. As a rule of thumb, it can be stated that thicker interfacial layers improve long-term stability (making low-molecular-weight emulsifiers less effective than proteins). The phospholipids occurring naturally in cream may therefore impact negatively on emulsion stability. Even within the class of milk proteins, marked differences occur in thickness of the interface: typical surface coverages for sodium and potassium caseinates and α- and β-casein are $\sim 2-3\ \mathrm{mg/m^2}$, whereas they are $\sim 10\ \mathrm{mg/m^2}$ for casein micelles (Pelan and others 1997). These differences in surface coverage should be explained as differences in interfacial thickness, and indeed sodium-caseinate-based emulsion gels are found to be less stable against (partial) coalescence during storage than emulsions made with casein micelles. The interfacial rheological properties of protein-based

interfacial layers provide an additional advantage over low-molecular-weight emulsifiers. The elastic properties of the proteins at the interface (Mellema and Isenbart 2004) also tend to prevent coalescence of the dispersed phase.

Proteins sometimes compete with fat crystals at the oil–water interface. The solid fat content in the lipid phase is known to influence the adsorption of casein proteins at the oil–water interface, as is illustrated by the decreased caseinate surface coverage in sodium-caseinate-stabilized emulsions stored below 15°C (Euston and Mayhill 2001), conditions under which large numbers of milk-fat crystals form. These crystals may induce partial coalescence as well. In this context, it should be noted that the homogeneity of the interfacial layer is important too: Denatured aggregated whey protein does not stabilize the emulsion very well (Kiokias and others 2004a; Kiokias and Bot 2005, 2006).

Emulsion Droplet Aggregation Under Neutral Conditions. Protein-stabilized oil droplets in the neutral premix may occur as either stable individual droplets or aggregated droplets (Fig. 28.6). The aggregated states are best described in terms of an interaction potential between droplets that takes into account both the electrostatic and steric interactions between protein-covered oil droplets. The interaction potential predicts attraction at short distances (short-range: van der Waals, hydrophobic), repulsion at long distances (long-range: electrostatic), and a local minimum at intermediate distances caused by steric repulsion (or interfacial elasticity) at short distances (Fig. 28.6) (Walstra and others 1996).

As a result, three states can be distinguished. If two droplets are stable and hence separated over long distances, a slight electrostatic repulsive force ensures that the droplets will remain separate. If two droplets are flocculated, and separated over intermediate distances, the attractive forces become larger than the repulsive forces and the oil phases are only prevented from touching by the protein layer covering them. The local minimum in the interaction potential is shallow and aggregation is reversible under shear. The state is referred to as clustered if the local minimum in the interaction potential is deep and aggregation is essentially irreversible, even under shear. If the short-range steric repulsion is overcome, the oil phases touch and either coalesce or partially coalesce/clump. Full coalescence is not a very common phenomenon in protein-stabilized systems, but

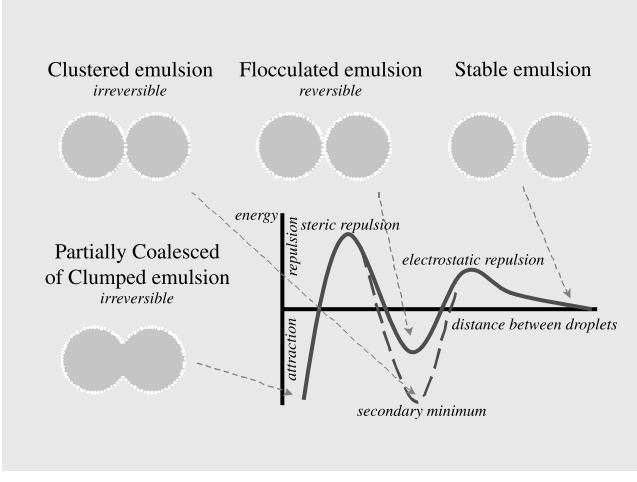

Figure 28.6 *Schematic representation of various emulsion states.*

partial coalescence may occur if the droplets have some firmness and/or contain (anisotropic) particles that are too large to be kept in one droplet, for example, upon crystallization of fat in the dispersed oil phase.

As electrostatic repulsion depends on the charge on the milk proteins, lower pH (compared to neutral pH) and higher ionic strength will reduce the height of the electrostatic repulsive barrier. For the steric repulsive barrier, besides electrostatics ("salted brush"), factors like the interfacial elastic properties and the quality (homogeneity) of the protein layer around the oil droplet are important.

Fat Structuring. As explained above, filler particles play an important role in composite properties, such as firmness and fracture properties. Concentration, size, and filler–matrix interaction effects are, however, greatly dependent on the physical properties of the particles, which are in turn linked to their crystallization behavior. Little is known about crystallization of dispersed fat in multicomponent and multiphasic systems like food products (Rousseau 2000), in contrast to crystallization in bulk oils. A better understanding of how fat behaves in such systems would open interesting perspectives to control droplet hardness and therefore composite properties (Kloek 1998).

The TAG crystallization mechanism in emulsions differs considerably from bulk crystallization. The process of crystallization consists of three stages: supercooling (or alternatively, supersaturation), nucleation, and crystal growth (Boistelle 1988). Crystallization in bulk occurs through heterogeneous nucleation involving trace impurities that provide the initial nucleation sites. Once crystallization starts, it rapidly spreads throughout the whole system by means of crystal growth and/or secondary nucleation (Sato 1988; Sato and Garti 1988; Timms 1994; Berger 1997). In emulsions, however, the lipid phase is finely dispersed in droplets and the number of impurities may be significantly less than the number of droplets, depending on the droplet size distribution in the emulsion. As a result, a considerable fraction of the fat droplets may be nucleus-free and their crystallization should occur through an alternative mechanism, such as the spontaneous formation of crystals of sufficient size to not dissolve again, known as homogeneous nucleation (McClements and others 1994; Davies and others 2000; Campbell and others 2002).

Homogeneous nucleation usually takes place well below the thermodynamic melting point, under conditions where the TAG mixture is a supercooled, metastable liquid (i.e., crystallization is thermodynamically favorable, but the required activation energy is high compared to the energy of the thermal fluctuations). Deeper cooling enhances the homogeneous nucleation rate, and therefore the fat phase in a larger fraction of the droplets will crystallize.

Alternatively, the interface can play a role. O/w emulsions contain a large interfacial area and the presence of (low-molecular-weight) emulsifiers may affect the crystallization kinetics by means of surface-induced heterogeneous nucleation as well. Emulsifiers decrease interfacial tension, making it more thermodynamically favorable for nuclei to form at the interface than in the interior of the droplets. Hence, the surface acts as a catalytic impurity promoting nucleation (Campbell 1989; Krog and Larsson 1992). This mechanism is not expected to play an important role in cream cheese, because low-molecular-weight emulsifiers are usually not added.

Finally, interdroplet heterogeneous nucleation may occur in situations in which nucleation is initiated in supercooled liquid droplets by contact with crystalline droplets of the same material, for example, crystals protruding from solid droplets and penetrating into

liquid droplets upon collision (McClements and others 1990, 1993a; Dickinson and others 1993). Interdroplet heterogeneous nucleation is promoted by high shear forces, low viscosity of the continuous phase, aggregated fat droplet state (*vs.* homogeneously distributed), high oil volume fractions, and ability of a crystal to penetrate the layer of emulsifier (possibly related to the interfacial modulus) (Mellema and Isenbart 2004). As proteins have been found to prevent interdroplet heterogeneous nucleation better than low-molecular-weight emulsifiers, this mechanism is not considered to be very important in cream-cheese-type emulsions.

Overall, the rate and degree of crystallization tends to be lower in fine o/w emulsions than in bulk, although the effect is not always large. This is illustrated in Figure 28.7, which shows a slightly lower solid fat content (expressed on fat phase) for whey protein-stabilized butterfat in oil emulsion and butterfat bulk crystallization. Incomplete crystallization will lead to lower firmness of the emulsion gel. Deeper cooling will bring the degree of crystallization in the emulsion closer to the bulk value.

28.2.2.2 Emulsion Structuring by Controlling the Continuous Phase. In composite systems like protein-stabilized emulsion gels, the continuous aqueous phase consists of a mixture of milk proteins, sugars, and salts. The textural properties of the final product are largely determined by (1) the physicochemical properties of the ingredients, (2) the order of addition, and (3) the applied processes, such as heating, acidification, and cooling.

Gelling of Milk Proteins. Casein micelles can form gels through acidification or renneting. Renneting, which induces splitting of the κ-casein at the surface of the micelle, is the basis of hard cheese formation and will not be discussed here. Acidification below ~pH 5 leads to sufficient charge neutralization in the κ-casein polyelectrolyte brush at the micelle surface to induce aggregation. The exact pH at which aggregation occurs depends on the amount of serum casein present, and therefore on temperature and the temperature–pH route taken to the point of destabilization (Vasbinder and others 2003). During acidification, the internal micellar structure weakens by the dissolution of the calcium phosphate.

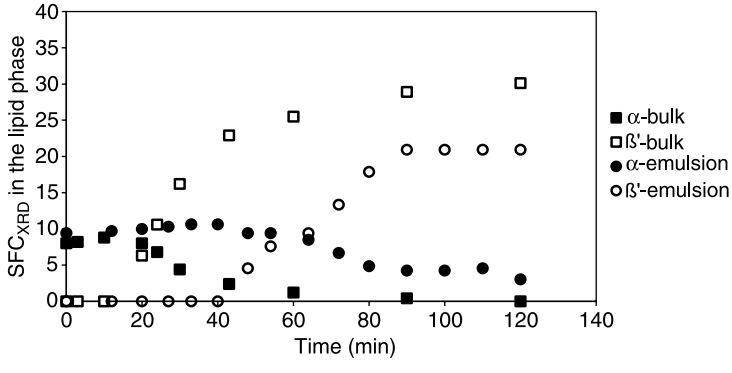

Figure 28.7 *Crystallization in 30% butterfat o/w model emulsion stabilized by pre-denatured whey protein from WPC (analysis using X-ray diffraction; filled and open symbols are derived from α and β′ polymorph peak intensities, respectively; squares and circles refer to crystallization experiments in bulk and emulsion, respectively). The comparison between crystallization in emulsion to bulk shows that modest supercooling occurs in the emulsion.*

For nonheated acid milk gels, typically a maximum in elastic modulus is found near the isoelectric point of the casein micelles (~pH 4.8), with a considerably steeper change in modulus above than below the isoelectric point. The maximum usually coincides with a steep change in water exudation from the gel, syneresis, which is much higher above the isoelectric point (Roefs and others 1990).

The mechanical properties of the casein gel are not so much determined by the mechanical properties of the micelles themselves, which are quite firm (Uricanu and others 2004), but rather by strands of micelles and thus by the properties of the connections between the micelles (Mellema 2000). The properties of the gels, such as texture and water binding, are influenced, for example, by casein concentration, presence of salts, shear forces, heat treatment, and rate of acidification. For example, increasing the rate of acidification results in gelling at a lower pH and in the formation of smaller and denser aggregates, which favors syneresis in pure casein systems. Casein gelling is discussed in more detail elsewhere (de Kruif 1998, 1999; Mellema 2000; Mellema and others 2000).

Whey proteins usually do not gel in their native state, but only after denaturation and at sufficiently high concentration. Gelling behavior of whey proteins generally follows the gelling of the main protein component, β-lactoglobulin. Upon denaturation, β-lactoglobulin exposes a reactive thiol group, which can act as an initiator for a "polymerization" reaction, during which aggregates are formed (Kella and Kinsella 1988; Kinsella and Whitehead 1989; Roefs and de Kruif 1994; Surroca and others 2002). Note that the rate of unfolding of the whey proteins can be delayed by environmental factors like the presence of lactose.

Casein and whey are present as a mixture in dairy products, which affects their gelling behavior, especially if combinations of heat treatment and acidification are applied. Upon heating at temperatures above 70°C, whey proteins denature and bind to casein micelles via hydrophobic and disulfide interactions with the κ-caseins. Heating skim milk at 90°C over 10 min leads to 40% coating of casein micelles by whey proteins (Vasbinder and others 2001), but details of the interaction depend strongly on temperature and pH (Vasbinder 2002). In milk, both β-lactoglobulin and α-lactalbumin bind to the micelle, the interactions between casein and β-lactoglobulin being limited by the number of binding sites available on the micelle surface, and the interactions between casein and α-lactalbumin being rather limited by its concentration in milk (Corredig and Dalgleish 1999). Because of this, increasing the whey/casein ratio may not necessarily result in a higher amount of whey being bound to the micelle.

The presence of denatured whey at the micelle surface increases gel firmness (Lucey and others 1998, 2000) and decreases the gelling time and increases gelling pH, because denatured whey proteins act as bridging agents between aggregating casein micelles during acidification. The resulting gel also has a more dense structure (Vasbinder and others 2001). The whey protein coating can prevent disaggregation of the micelles. Obviously, the properties of such a mixed gel are dependent on the degree of whey denaturation, with accompanying consequences on textural properties and syneresis. It appears that in heated milk gels, the reduction in elastic modulus and the decrease in syneresis below the isoelectric point of the casein micelles become less pronounced or shift to lower pH (Xiong and others 1991).

Emulsion Droplet Aggregation Under Acid Conditions. Under acid conditions like in cream cheese, the electrostatic repulsive barrier between the protein-stabilized droplets is

lowered and thus the droplets will aggregate (Fig. 28.6). Depending on the homogeneity of the protein layer around the emulsion droplets, the emulsions can be characterized as either flocculated/clustered or coalesced/clumped. An essentially flocculated emulsion is prepared if a strong protein film around the droplets is first made (e.g., by heating a properly stabilized emulsion). A clumped emulsion can be prepared from emulsions with much crystalline fat if coarse protein aggregates are first made by denaturation, followed by emulsification (Kiokias and others 2004a).

Partial coalescence is an interesting phenomenon in composite gels because the formation of a fat network by bridging of crystals from one droplet to another may help to modify textural attributes, like improved mouthfeel (Boode and Walstra 1993; Gelin and others 1994; Campbell and others 1996; Pelan and others 1997) or defects like hardening upon storage (Kiokias and others 2004a). For high-volume-fraction emulsions, like cream cheese, partial coalescence upon temperature cycling does not depend on droplet collision frequencies, because the droplets are always in close contact.

The structure of the droplet aggregates is expected to be important, but unfortunately very difficult to measure. Preliminary studies on model systems using Spin Echo Small-angle Neutron Scattering (SESANS) were done to address this (Bot and others 2006; Bot and others 2007). A priori it is expected that fine homogeneous droplet networks will result in firmer networks, much the same as in protein networks.

The mechanical properties of the emulsion gel follow the characteristics of the matrix, as was demonstrated for the behavior of an acid emulsion gel and a comparable acid milk gel as a function of pH (Xiong and others 1991). Similar trends are expected to hold true for syneresis in acid emulsions.

28.3 CREAM CHEESE PROCESSING

The traditional cream cheese production process consists typically of the following steps. A mixture of pasteurized, homogenized milk and cream is acidified via fermentation using lactic acid bacteria, which leads to aggregation of the milk protein. The aggregated milk protein, including the fat droplets contained in the protein matrix, is concentrated by separating off a liquid phase through mechanical treatment (usually centrifugation), if necessary combined with a heat treatment. The concentrated phase, the curd, can be pasteurized to inactivate the lactic acid bacteria and is usually homogenized once more before the product is filled in the final packaging (Guinee and others 1993). Usually, a sealed container and chilled storage are used to increase the shelf-life of the product. More details are provided in the following sections.

The production process is complex from a physicochemical point of view due to the presence of two irreversible steps, milk protein aggregation during acidification and separation of the whey from the curd. In addition, acidification is performed by live organisms, introducing some variability in the process.

28.3.1 Emulsification

As cream cheeses are emulsions, it is necessary to mix the dispersed phase thoroughly into the continuous phase. The efficiency of the emulsification stage depends predominantly on the power input during the emulsification process and on the interfacial tension between lipid and water phases. The proteins in the premix reduce the interfacial tension, and therefore facilitate the emulsification process. Emulsification in factory-scale cream cheese

production is achieved by intense mechanical shearing of the premix of milk and cream, usually with a high-pressure homogenizer. A homogenizer is essentially an orifice or a valve that blocks the fluid flow, causing intense shearing forces as the liquid is forced through the narrow gap. Dispersed phase droplet sizes below 1 μm can be obtained with such equipment when operating at a pressure above ~100 bar. Reduction of the dispersed liquid droplet size becomes increasingly difficult for small droplets, because the surface area in an emulsion with droplets of radius R increases as $1/R$.

Droplet sizes after homogenization can be measured routinely through low-field NMR techniques directly in the product (Goudappel and others 2001; Kiokias and others 2004b) or by static light scattering in diluted o/w emulsions (Gelin and others 1994; Bot and others 2003b).

A special phenomenon called homogenization clustering occurs in emulsions stabilized mainly by casein micelles (such as a cream cheese premix). Because micelles tend to diffuse relatively slowly, the newly created droplet interface is sometimes not covered rapidly enough by micelles from the serum phase in the break-up phase. If droplets meet again in the zone beyond the homogenization valve, this may result in a situation in which micelles stabilize two droplets at the same time and clusters of micelle-coated fat droplets are formed. This situation was already outlined in Figure 28.6, and will occur only for emulsions stabilized by relatively large particles, such as casein micelles. Homogenization clustering may lead to an increase in viscosity.

28.3.2 Acidification

Acidification is normally achieved by fermentation, which generates a wide range of subtle taste and flavor components enhancing the dairy connotation. Fermentation can either be done by thermophilic microorganisms, which show optimal activity at ~40°C, or by mesophilic microorganisms, which prefer temperatures of ~25°C. Thermophilic organisms tend to give relatively acidic yogurt-type flavors, whereas mesophilic organisms generate a wider, more subtle range of flavors. The latter tend to require longer fermentation periods, but are usually applied in cream cheese production.

Fermentation is usually performed in a batch process, but alternatives have been considered. For example, it is possible to design a continuous fermentation process in which neutral mix is added continuously to the fermentation tank, and acid mix is tapped from the tank. However, such processes have not been widely introduced as a standard because in-line control of the pH in an industrial process is notoriously difficult. Furthermore, the stability of commercial multistrain fermentation cultures over prolonged periods is not guaranteed as a result of competition among the strains.

Disadvantages of fermentation are the long periods required to achieve acidification, the fact that the period required for fermentation is never completely predictable, and the sensitivity of the fermentation cultures to phage infections.

28.3.3 Separation

The aggregated milk protein, including the fat droplets contained in the protein matrix, is concentrated by separating off a liquid phase. This allows the formation of a cream cheese with firm texture, high in milk protein but without taste defects associated with high mineral (calcium) or lactose level. Depending on the fat/protein ratio of the product, a cream-type separator or a quark-type separator may be used. Higher temperature may

be applied to facilitate the separation of the whey from the curd. Usually, it is found that the amount of dry matter in the curd decreases with decreasing pH in the pH range below the isoelectric point (Kim and Kinsella 1989).

If the milk has not been heated to denature the whey proteins, most of these proteins will still be soluble in the liquid phase and will therefore disappear with the waste stream. As an alternative to separation one could consider ultrafiltration, in which the whey protein is retained in the curd.

Before filling the curd into the final packaging, an additional high-pressure homogenization step is often applied to break up the larger protein aggregates and give the product a smoother texture.

28.3.4 Filling

Cream cheese is a relatively sensitive product in terms of microbiological stability, but the product keeps quite well if general hygienic requirements for the processing line and factory are met (see, for example, Lelieveld 1999). The product should be pasteurized, brought to a sufficiently low pH, and hygienically packed in a sealed container.

An important decision concerning process design is whether the products will be filled using hot filling or cold filling. Hot filling usually means filling the product at pasteurization temperature, sealing it, and thus using the product to pasteurize the packaging. The disadvantage of this process is that the product has to be cooled down to chilled storage temperature in the final packaging. This is a slow process and may induce product defects (e.g., condensation of water in the headspace above the product). Cold filling does not introduce these problems, but requires higher standards in packaging hygiene and in-line cooling of the product. In-line cooling (as well as heating) is usually carried out in tubular, plate, or scraped surface heat exchangers.

28.4 CREAM CHEESE PROPERTIES

The taste and textural properties of cream cheese products that are relevant for consumers may to a large extent be attributed to its composite microstructure. Important aspects are spreadability, melting in the mouth, and syneresis.

28.4.1 Spreadability

A spreadable texture can only be obtained if the product is built from microstructural elements that interact weakly and reversibly, resulting in a more or less plastic rheology. Very loosely stated, a plastic material is a solid-like material for which the deformation history (e.g., flow experienced) does not affect its current mechanical properties. In this sense, a plastic material is reminiscent of a liquid. A typical solid (e.g., a chocolate bar) would break upon application of sufficient deformation and never regain its original properties.

Few materials are really plastic, according to this description, but butter is a reasonable approximation (de Bruijne and Bot 1999; Bot and others 2003a). Cream cheese is plastic in the sense that it is built from microstructural elements (protein/fat) that interact weakly. However, the interactions between the proteins are generally not reversible and plasticity will generally be higher in products where the fat/protein ratio is higher. Besides plasticity, product hardness is of course an important factor determining the spreadability of cream cheese.

28.4.2 Melting in the Mouth

The qualitative data in Figure 28.4*a* has already indicated that the dispersed phase contributes considerably to the firmness of the product, and therefore it is logical that the melting of the butterfat in the mouth helps to soften the product. It should be realized, however, that the loss of firmness is not as extreme as in a product like butter (de Bruijne and Bot 1999; Bot and others 2003a). In butter, the continuous phase melts completely, whereas in cream cheese a considerable contribution to the viscosity can still be expected to come from the protein phase. Both the melting and the residual firmness/viscosity are illustrated in Figure 28.5. It is shown that indeed much of the firmness is lost during heating to mouth temperature, but above $\sim 30^{\circ}$C, the firmness is mostly determined by the protein network and further melting of the fat phase does not reduce the firmness any further. The end viscosity could be adapted by using more or less protein.

28.4.3 Syneresis

In a milk protein gel, 90% of the water is enclosed in the pores between the casein strands forming the network and 10% within the casein particles (van Vliet and Walstra 1994). Exudation of part of this water phase from the gel upon collapse and/or contraction of the gel matrix, a phenomenon called syneresis, occurs in most (particularly particle) gels. The process proceeds most rapidly in coarse inhomogeneous protein networks, which can be formed over time as a result of local reorganization in the milk gel (Mellema 2000). Semiquantitative descriptions of the process are based on the Darcy equation and involve the viscosity of the water phase and the geometry of the protein network through which the water phase flows (Darcy 1856; Bird and others 1960; Lucey and others 1997; de Bruijne and Bot 1999; Mellema and others 2002). The mechanisms promoting exudation of the water phase are thought to be rearrangements of the protein network ("endogeneous" exudation) and buoyant forces on continuous phase (due to density differences between exuding phase and network). In specific geometries, drainage is a possible mechanism too, for example, when a product is positioned on a grid or into the void where the product is scooped out of a consumer package.

Syneresis during cream cheese production is desirable (and can be accelerated by stirring, heating, and centrifugation). In final cream cheese products, however, syneresis is considered to be a defect, only allowed to occur to a limited extent.

There are various ways to decrease syneresis.

1. A finer, more homogeneous network has fewer large pores and therefore less syneresis. Although it is difficult to formulate a general rule because many factors play a role in network formation, this can usually be achieved by gelation at pH values away from the isoelectric point pI (due to enhanced rearrangements near pI), lower temperatures (hydrophobic interactions between caseins at higher temperatures induce aggregation), slower acidification (larger flocs as building blocks for the gel). For systems based on heated milk, however, it should be realized that the "critical" pH can actually be somewhat lower than the pI, for example, pH $\sim$ 4.
2. A firmer network tends to suffer less from rearrangements at micellar length scales, and therefore shows less syneresis. Denaturation of the whey proteins upon heating of the milk has been shown to reduce syneresis compared to unheated gels (Augustin

and others 1999; Dannenberg and Kessler 1988a,b; Lucey and others 1999, 2000), possibly due to covalent disulfide bonds and increased stability of the micelles against rearrangements.

3. An increased viscosity of the water phase reduces syneresis, for example, through the addition of low amounts of polysaccharides (Bot and Vervoort 2006).

28.5 CREAM CHEESE PROPERTIES DURING TRANSPORT AND STORAGE

During transport and storage of cream cheeses a number of undesirable changes may occur. These changes are partly due to the fact that cream cheese texture still develops after leaving the production line (fat crystallization, protein network formation) and partly to temperature fluctuations during storage and transport.

For a fresh cream-cheese-type product, microbiological stability during storage and transport is often the most critical property. To extend the shelf-life of the product before opening by the consumer ("closed shelf-life"), chilled transport and storage are usually preferred. Improper transport and storage conditions at higher temperatures for longer periods may affect keepability. Additionally, syneresis may become unacceptably high and taste may be affected (for example, by oxidation processes).

Cream cheese products also tend to be temperature cycled during consumer usage (in and out of the fridge). Sometimes this leads to partial coalescence of the fat droplets in the protein matrix, which may result in considerable post-firming of the emulsion gel. Partial coalescence may also coarsen the pores of the protein network during temperature cycling, once again leading to increased syneresis compared to noncycled samples. Acidified whey-protein-stabilized o/w emulsions based on partly crystalline fat, in which the functionality of the whey protein has been impaired before emulsification by ("pre-") heat-treatment and subsequent aggregation, form interesting model systems to investigate this effect (Kiokias and others 2004a). Such emulsions can destabilize at relatively high protein content, even under conditions at which their native neutral counterparts are still very stable.

28.6 SUMMARY

Cream cheese products are protein-stabilized emulsion gels created from water, fat, and proteins. The main processing unit operations are homogenization (emulsification), heating (pasteurization and protein denaturation), acidification (protein gelling), and cooling (fat crystallization). A broad range of microstructures can be formed with all potential composition/processing combinations. This chapter gave an overview of the basic phenomena that are involved in the production of cream cheese. The main phenomena of interest specifically relevant for the texture of cream cheeses were gel firmness, stability (syneresis of casein gel), denaturation (of whey proteins), and partial coalescence (of fat droplets).

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Section VIII

Confectionery

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29

Chocolate and Cocoa

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29.1 Origin 676
29.2 Cocoa Bean Production 676
29.3 Cocoa Bean Processing 677
29.4 Cocoa Powder Manufacturing 678
29.5 Chocolate Manufacturing 682
29.5.1 Ingredient Mixing 682
29.5.2 Refining 682
29.5.3 Conching 683
29.5.4 Viscosity 683
29.5.5 Tempering 684
29.6 Production Methods 686
29.6.1 Enrobing 686
29.6.2 Cooling 686
29.6.3 Depositing and Molding 687
29.6.3.1 Depositing 687
29.6.3.2 Molding 687
29.7 Confectionery Coatings 688
29.7.1 Compound Coatings (Vegetable Fat Coatings) 688
29.7.2 Sugar-Free Coatings 689
29.8 Chocolate Bloom Problems 690
29.8.1 Sugar Bloom 690
29.8.2 Fat Bloom 690

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

29.9 Storage and Handling 691
29.10 Nutritional Value 691
References 693

29.1 ORIGIN

Chocolate is one of the most popular flavors in the world. It is widely used in many food products, including soups, main dishes, beverages, baked goods, and of course confectionery. Its basic ingredients are chocolate liquor, sugar, cocoa butter, and flavors. Other ingredients such as herbs, spices, milk, nuts, crisp rice, and so on, may also be added.

Under conditions of semishade, warmth, and high humidity, cacao trees (*Theobroma cacao*) have thrived and been cultivated in the Americas for at least an estimated 4000 years. It was important in Mayan and Aztec mythology and also used as currency. Originally, an unsweetened foamy beverage called *chocolatl* was served to the noble ruling class. Early Spanish explorers found that adding sugar to this bitter *chocolatl* liquid improved its flavor and soon became the rage of the Spanish nobilty. This sweetened *chocolatl* liquid then spread to Italy, Holland, France, and England.

However, this sweetened *chocolatl* beverage was very rich in fat and difficult to disperse in water. Some consumers also had difficulty in digesting it. In the early 1800s, C.J. Van Houten of Holland patented a cocoa press that could separate chocolate liquor into cocoa butter and its residue, which is processed into cocoa powder. Mr. Van Houten was also credited with inventing the Dutch Process, by alkalizing the cocoa to give it a darker color and a less acid flavor. It was found that a superior product could be made with cocoa powder when warm water replaced the cocoa butter.

Fry and Son in 1847 and Cadbury in 1849 were among the first to sell solid eating chocolate with a basic formula of chocolate liquor, sugar, and cocoa butter. The development of solid chocolate was made possible when the separation of cocoa butter from chocolate liquor resulted in an "excess" of cocoa butter. The addition of cocoa butter to chocolate liquor and sugar resulted in a smooth and creamy product. In 1875 Daniel Peter developed a method of adding condensed milk to chocolate and thus milk chocolate was born.

29.2 COCOA BEAN PRODUCTION

There are three major types of cocoa (cacao) beans: Forastero, Criollo, and Trinitario. The region where the cocoa beans are grown and its variety can greatly affect its characteristics for flavor, color, hardness of butter, and so on. Forastero beans are generally dark brown in color with a strong, bitter flavor and account for the majority of the beans. Criollo beans are the flavor beans. They are lighter in color with a mild, nutty flavor. Trinitario is basically a cross between Criollo and Forastero. The beans are blended to produce the desired end product.

Cacao trees are commercially cultivated generally within a 20° latitude of the equator with a rainfall of 45–100 in. (114–254 cm) and a temperature of 70–90°F (21–32°C). Africa (Ghana, Nigeria, Ivory Coast), South America (Brazil, Ecuador, Venezuela), the West Indies (Dominican Republic), Asia and Oceania (New Guinea, Malaysia, Indonesia) are the major producing areas.

A cacao tree will attain its full height in about 10 years. Although trees may grow to 40 ft (1200 cm) in height, they are usually pruned to 15–25 ft (450–750 cm) to facilitate harvesting. Flower clusters appear only on the trunk and main branches, with 20 to 30 fruit pods developing about five to six months later. The growth cycle is continuous, so a tree may bear leaves, blossoms, and pods simultaneously. However, the main fruiting season occurs between October and February.

The ripe pods are elliptical in form, 7–10 in. (18–25 cm) in length and 3–5 in. (8–13 cm) in diameter, containing approximately 20–50 seeds surrounded by a mucilaginous pulp. Each dried cacao bean weighs approximately 1 g. When dried, the beans from each pod weigh between $1\frac{1}{2}$ and 2 oz (42–56 g). Although yields can vary greatly, the average tree produces 20–30 pods, so each tree's output is between 2 and 3 lb (0.9–1.4 kg) of commercial cocoa beans.

Mature cacao pods turn yellow, orange, or purple in color. The pods are split open and the beans and pulp scooped out and heaped into boxes or baskets for fermentation (anaerobic and aerobic). The beans are allowed to ferment for 3–6 days, depending upon the type of beans used, batch size, temperature, and aeration. Aeration (turning) of the beans promotes bacterial activity and ensures uniform fermentation. Fermentation and drying processes have major influences on the quality of the beans used in making chocolate. The beans are subsequently dried (naturally or mechanically) to a moisture content of less than 8% to prevent mold growth, with the optimum being 6–6.5%.

Cacao beans must be cleaned before processing to produce a wholesome product with minimal microbiological risks and to remove extraneous materials. On a dry basis, the beans are approximately 87.1% nib (cotyledons), 12.0% shell, and 0.9% germ. The nib contains about 55% cocoa butter fat (Hofberger 1999b).

Cocoa beans arriving in the United States are usually inspected for mold, infestation, filth, degree of fermentation, and bean size. They are also fumigated at the ports when received. They should be stored in cool, dry, and well-ventilated warehouses to maintain quality.

29.3 COCOA BEAN PROCESSING

There are several options on how to process the cocoa bean. They all involve the removal of the shell and a roasting step. The roasting process results in the development of the flavor and aroma of the beans. There are several methods, which includes whole bean, nib, and chocolate liquor roasting. Roasting can take anywhere from 15 min to 2 h. Whichever roasting method is used, the nibs are separated from the shell (winnowing). Depending upon the degree of roast, the final nib moisture ranges from 1.5–3%.

The traditional method of roasting involves the whole bean. The beans enter a gas-fired revolving drum with various air flows and a cooling section. Temperature and length of roast will depend on equipment, flow rates, type of beans, size of beans, and the flavor desired. Cooled roasted beans will then go through a cracking step to break the nib into large pieces. Winnowing machines will use density differences and sieving to separate the nibs from the shell. Federal standards allow no more than 1.75% shell in the nib portion used for chocolate production. Cocoa-bean shells have little commercial value and are used for mulches, fertilizer, fuel, bedding, and in some cigarette blends. The germ portion is also removed because it is hard and gritty.

A common approach to roasting cocoa beans is to rapidly heat the exterior of the beans with infrared heat. The shell expands from the unheated nib portion, where it is easily removed by winnowing. The nib is then roasted in a similar manner to the whole cocoa beans. Several advantages to using this method include lower microbiological count, less fat migration to the shell portion, and better separation between the shell and nib.

The third method of roasting involves grinding raw cocoa nibs into a liquor. This liquor is then heat treated (i.e., roasted) at various times and temperatures to produce the desired finished product. This procedure is said to result in a product with uniform flavor and color. Unlike the two previous methods where large, small, and broken beans may be over- or under-roasted due to size differences, the liquor roasting method has one common heat treatment.

After roasting, the nibs are ground to a liquid state called chocolate liquor. The heat and friction from the grinding process will rupture the cell walls of the nibs to release the valuable cocoa butter. This liquor (containing approximately 55% cocoa butter) will solidify upon cooling. Nib grinding carried out using several methods, with most manufacturers using a combination of methods to obtain the desired particle size with the maximum available cocoa butter.

Micropulverizers (often called hammer mills) are the commonly used first step in the grinding process. Although they can be used to produce fine particle size liquor, other mills are often used for final size reduction. This final degree of fineness is important to maximize the amount of free cocoa butter available for viscosity reduction in chocolate, pressing of liquor into cocoa cake and butter, and particle size of the cocoa powder.

Stone mills are commonly used for final particle size reduction. They generally have three pairs of grooved "stones", with each pair progressively making the liquor finer. Each pair of stones has a stator and rotor that can be adjusted in distance. Liquor enters the center of the stones and discharges at the outer circumference to the next pair of stones.

A fine particle size may also be obtained with a ball mill. This equipment consists of a tank containing small metal or ceramic balls. Pre-ground liquor enters the bottom of the vertical tanks and is reduced in size by the rotating motion of an agitator on the balls.

A five-roll refiner is the final method that is used to reduce particle size. The ground nibs are introduced to the lower roller and move up due to the rollers rotating in alternating sequence with increasing speed.

The processing for cocoa and chocolate are essentially the same in the initial stages. The beans are cleaned, roasted, and shelled. The manufacturing of cocoa and chocolate, however, involve two separate processes (Fig. 29.1).

29.4 COCOA POWDER MANUFACTURING

Cocoa cake and cocoa butter are separated from the hot chocolate liquor by hydraulic pressing (more than 6000 psi). This cocoa butter, flowing out from the press, contains small amounts of cocoa powder, which are filtered out to maintain consistent color and flavor. The butter may be further refined, bleached, and deodorized (via steam distillation) to yield a very bland, clean-tasting cocoa butter. What is left in the

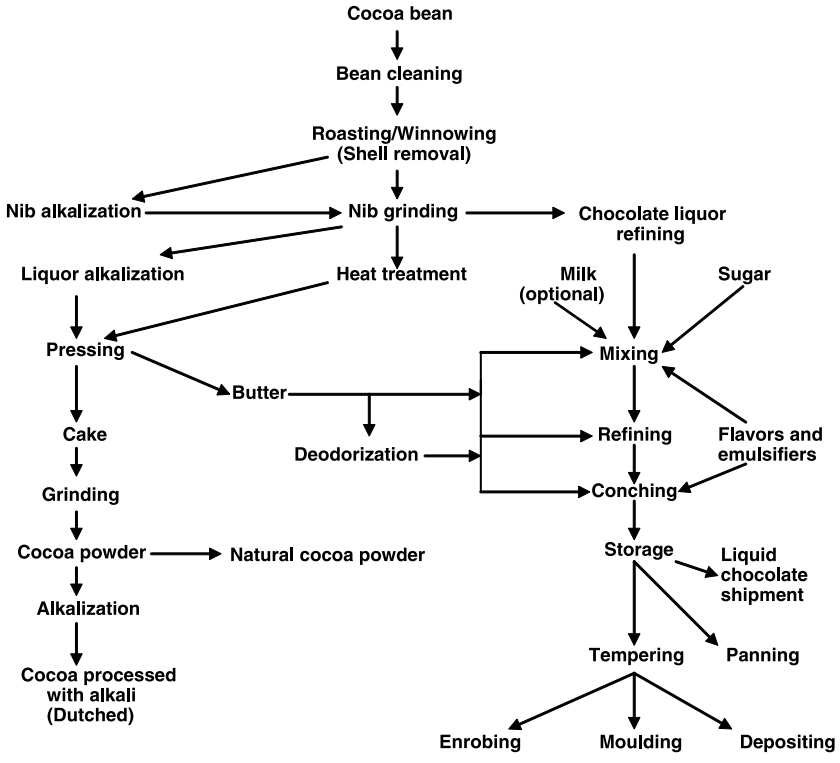

Figure 29.1 *Manufacture of cocoa and chocolate.*

press is a cake of cocoa powder (cocoa cake). The cocoa cake will generally have a fat content ranging from 10–24%. Cocoa powder is the cake ground to the desired degree of fineness – usually as 95–99% will pass through a 200-mesh screen. Powders with less than 10–12% cocoa butter are known as low-fat cocoas. Medium-fat cocoa contains 10–18% fat, and high-fat or "breakfast cocoa" has 22–24% fat. Approximately 95% of the cocoa powders on the market today are the medium-fat variety. The FDA has established Standards of Identity for various types of cocoa products (Table 29.1).

Natural cocoa is cocoa powder that has not been treated with alkali. Van Houten, in 1828, first treated cocoa with the addition of alkali; hence, it is also known as "Dutch" process cocoa. The alkalization process raises the pH of the cocoa powder from 5.2–5.6 in fermented beans to a pH of 6.8–7.5. Black cocoas, used for color, may have a pH as high as 8.5 (Minifie 1989c). FDA standards allow a maximum of 3.0% potassium carbonate per 100 lb (45 kg) of nib weight equivalent. Added alkali neutralizes acidity in the cocoa, resulting in a milder, less astringent flavor and a range of colors (from light brown to red and even black). Various formulas, equipment, and processes are used to alkalize the nibs, liquor, or powder. This allows for a wide range of cocoa powders to be produced. As such, alkalized cocoa powders are in greater demand than natural cocoa powder. Table 29.2 lists the various cocoa powder types and their applications.

TABLE 29.1 U.S. Standard of Identity for Cacoa Products, Code of Federal Regulations (CFR), Title 21, Food and Drugs.

21 CFR Reference	Product	Chocolate Liquor (%)	Milk Solids (%)	Comments
163.111	Chocolate liquor	100	0	• 50–60% fat • Optional ingredients 1 • Other names: unsweetened chocolate, bitter chocolate, baking chocolate, cooking chocolate
163.112	Breakfast cocoa		0	• 22% fat (min) • Optional ingredients 1, with the exception of butter or milk fat
163.113	Cocoa		0	• 10–22% fat • Optional ingredients in 163.112 • Other name: medium-fat cocoa
163.114	Low-fat cocoa		0	• Less than 10% fat • Optional ingredients in 163.112
163.123	Semisweet/ bittersweet chocolate	35 (min)	12 (max)	• Sweetened chocolate liquor, which may contain one or more, specified optional ingredients 2 • Traditional bittersweets 50% or more liquor
163.123	Sweet chocolate	15–35	12 (max)	• Sweetened chocolate liquor, which may contain one or more, specified optional ingredients 2
163.124	White chocolate	0	14 (min)	• 20% cacao fat (min) • 3.5% milk fat (min) • 55% nutritive carbohydrate sweetener (max)
163.130	Milk chocolate	10 (min)	12 (min)	• Sweetened chocolate liquor with dairy ingredient(s), which may contain one or more, specified optional ingredients 2
163.150	Sweet cocoa and vegetable fat coating		No limit	• Same as 163.123 except: mixture of cocoa and chocolate liquor contains a minimum of 6.8% nonfat cacao solids; contains one or more optional ingredients 3
163.153	Sweet chocolate and vegetable fat coating		12 (max)	• Same as 163.123 except that it contains one or more optional ingredients 3 (except for cocoa)
163.155	Milk chocolate and vegetable fat coating		12 (min)	• Same as 163.130 except that it contains one or more optional ingredients 3 (except for cocoa)

(*Continued*)

TABLE 29.1 *Continued.*

21 CFR Reference	Product	Chocolate Liquor (%)	Milk Solids (%)	Comments
				Optional Ingredients 1 • Optional alkalizing ingredients: ammonium, potassium, or sodium bicarbonate, carbonate, or hydroxide, or magnesium carbonate or oxide • Optional neutralizing ingredients: phosphoric acid, citric acid, and L-tartaric acid • Spices, natural and artificial flavorings, ground, whole nut meats, ground coffee dried malted cereal extract, and other seasoning that do not either singly or in combinations impart a flavor that imitates the flavor of chocolate, milk, or butter • Other optional ingredients: cocoa butter and cocoas, dairy butter or milk fat, salt When optional ingredients are added, it must be declared on the label.
				Optional Ingredients 2 • Optional alkalizing ingredients: ammonium, potassium, or sodium bicarbonate, carbonate, or hydroxide, or magnesium carbonate or oxide • Optional neutralizing ingredients: phosphoric acid, citric acid, and L-tartaric acid • Optional ingredients: cocoa butter, nutritive carbohydrate sweeteners • Spices, natural and artificial flavorings, ground, whole nut meats, ground coffee, dried malted cereal extract, salt, and other seasoning that do not either singly or in combinations impart a flavor that imitates the flavor of chocolate, milk, or butter • Optional dairy ingredients: cream, milk fat, butter, milk (concentrated, evaporated, sweetened condensed, dried), skim milk (concentrated, evaporated, sweetened condensed, nonfat), concentrated or dried buttermilk, malted milk • Other optional ingredients: less than 1% emulsifying agents When optional ingredients are added, it must be declared on the label.
				Optional Ingredients 3 • Cocoa, chocolate liquor • Safe and suitable vegetable derived fats, oils, or stearins other than cocoa butter (may be hydrogenated) • Safe and suitable dairy-derived ingredients, bulking agents, formulation aids, humectants, and texturizers
				Optional Ingredients 4 • Optional dairy ingredients: cream, milk fat, butter, milk (concentrated, evaporated, sweetened condensed, dried), skim milk (concentrated, evaporated sweetened condensed, nonfat), concentrated or dried buttermilk, malted milk; whey or whey products not to exceed 5% • Spices, natural and artificial flavorings, ground whole nut meats, ground coffee, dried malted cereal extract, salt, and other seasonings that do not either singly or in combination impart a flavor that imitates the flavor of chocolate, milk, or butter • Other optional ingredients: nutritive carbohydrate sweeteners, antioxidants, 1.5% or less emulsifying agents When optional ingredients are added, it must be declared on the label.

Source: U.S. Code of Federal Regulations. 2004a. Title 21, Part 163, 2004.

TABLE 29.2 Types and Applications of Cocoa Powder.

Cocoa Type	Flavor	Applications
Nonalkalized		
Natural (pH 5.2–5.7)	Mild, cocoa notes	Compounds, syrups, bakery toppings, confectionery
Alkalized		
Light Dutch (pH 6.5–7.0)	Mild, cocoa notes with mild alkali undernotes	Dairy, beverage, creams, syrups, toppings, confectionery
Red (pH 7.0–8.0)	Moderate to strong alkali notes	Bakery, dairy, beverage, cream, confectionery
Dark (pH 7.0–8.0)	Mild to moderate alkali notes	Bakery, confectionery
Black (up to pH 8.2)	Intensely unique alkali flavors	Cookies, coloring agent

Source: Adapted from Dyer (2003).

29.5 CHOCOLATE MANUFACTURING

Manufacturing of dark, milk, and white chocolates involves certain basic operations: ingredient mixing, refining, conching, standardization of viscosity, and tempering. Like cocoa powders, the chocolate liquor (white chocolate uses cocoa butter only) may undergo alkalization prior to further processing. The FDA has issued Standards of Identity for milk, dark, and white chocolate (Table 29.1).

Many chocolates seen on the market will advertise something like 60% cocoa solids. This is really cocao solids, which includes cocoa butter, cocoa powder, and chocolate liquor. In general, a more intense flavor occurs with a higher percentage of cacao solids.

29.5.1 Ingredient Mixing

Chocolate liquor, sugar, cocoa butter, milk products (for milk chocolate), emulsifiers, and flavors are the basic ingredients that are blended together. The result is a paste with a rough texture and plastic consistency. It is often passed through a kneader or plasticizer to improve uniformity prior to entering the conches.

29.5.2 Refining

In order to obtain a smooth texture with increased surface area, steel rollers are used to reduce particle size of the mass to 10–40 μm. This process is called refining. The actual size will depend upon the product desired and type of chocolate, with dark chocolate generally having a smaller finished particle size. During this process the resulting mass often turns into a dry paste or powder.

The manufacture of milk chocolate is similar to that of dark chocolate. The way milk is added plays an essential part in the process. Milk solids are introduced in the form of milk powder or milk crumb, where it is dry mixed with chocolate liquor and sugar. Milk powder is produced by first concentrating liquid milk and drying it into a powder. The crumb process involves blending sweetened condensed milk and chocolate liquor. It is kneaded and dried. Crumb-based chocolates have a unique caramelized flavor based on the Maillard reaction between milk protein and sugars (Minifie 1989e; Stauffer 2000).

The term "white chocolate" has been used loosely for a number of years. It is basically milk chocolate with cocoa butter but no chocolate liquor. Cocoa butter is mixed with sugar, milk solids (a white crumb can be made from cocoa butter), emulsifiers, and flavors. Use of a

mild-flavored cocoa butter is preferred because the flavor of white chocolate is quite delicate. White chocolate is also more prone to oxidative rancidity than milk and dark chocolate.

29.5.3 Conching

After refining, the mass is transferred to large shear mixers (conches). This is the last manufacturing process where texture and flavor are affected. Time, temperature, moisture control, and shear manipulate the process. Some of the benefits of conching are (Hofberger 1999b) as follows:

- Improved rheology/reduction in viscosity (less cocoa butter needed);
- Elimination of harsh volatiles for a mellower taste;
- Removal of moisture (reduces lumping and graining);
- Improved mouthfeel (smoothes sharp particle edges).

Additional cocoa butter, flavors, and emulsifiers may be added during this process. Conching times will vary depending upon the formulation and final product desired, but can vary from 10 to 12 h up to several days. Conching temperatures range from 120 to 160°F (49–71°C), and sometimes up to 180°F (82°C). The higher conching temperature gives the final product a caramelized flavor that is different from milk crumb caramelized flavor (Minifie 1989f).

29.5.4 Viscosity

Standardization of product viscosity is one of the final steps in the manufacturing of chocolate. The development of automatic molding and enrobing equipment requires precise control over the fluidity or viscosity of the chocolate. It should be noted that the viscosity of chocolate increases with the presence of *free* moisture in chocolate. Thus, general moisture tests will not necessarily provide pertinent information for viscosity. Minute amounts of water or steam from leaking equipment, improper storage (humid conditions), and rework can affect the overall performance of the chocolate.

Chocolate is a non-Newtonian liquid in that its viscosity (internal friction of fluids) is affected by the presence of solids in suspension, as well as by temperature. Once chocolate starts to flow, its viscosity will decrease with an increase in the shear rate (Minifie 1989d; Hofberger 2000).

The United States is the only country that still uses degrees MacMichael to decribe chocolate viscosity. The MacMichael viscometer is a single-speed rotational instrument. It works on the following principle: A metal cylinder is suspended on a torsion wire, which, in turn, is immersed in a cup of chocolate at a given temperature. As the cup rotates, twisting of the wire is measured by a scale. The main drawback of the MacMichael method is its inability to provide full information on the flow properties of different chocolates.

In other parts of the world, viscosity is usually measured in centipoise. A Brookfield viscometer can determine plastic viscosity and yield values accurately. Results using a Brookfield can be converted to degrees MacMichael. Table 29.3 shows a range of chocolate viscosities and their typical applications. Viscosity alone will not indicate how the product will perform in the handling process. Two terms that help to describe flow character are yield value and plastic value. Yield value (YV) is the force required to initiate the flow of chocolate. Plastic viscosity (PV) is the force required to maintain

TABLE 29.3 Chocolate Viscosities and Their Applications.

Viscosity (in °MacMichael)	Applications
Very thin (<100°MacMichael)	Enrobing, panning, shell molding
Thin (100–125°MacMichael)	Enrobing, panning, solid molding
Medium (126–160°MacMichael)	Enrobing, hand dipping
Heavy (161–200°MacMichael)	Depositing (chips), hand dipping, molding

Source: Adapted from Hofberger (2000), Wilbur Chocolate (2003).

the flow of chocolate once it has started to move. A high YV is important in maintaining decoration marks and the prevention of "feet" on enrobed goods. A low YV is desirable for molded products to properly shake out air pockets (Minifie 1989d; Hofberger 2000).

Several factors can affect viscosity and rheology. Smaller particle size in a constant formula will give a higher viscosity. Lecithin is thus an excellent emulsifier as it exhibits both lipophilic and hydrophilic properties. The hydrophilic groups attach themselves to the water molecules on the surface of the sugar particles and reduce friction, increasing particle mobility and thus lowering viscosity (Minifie 1989d). This reduction in viscosity can also decrease the need for more expensive cocoa butter by as much as 5% (Hofberger 2000). In the United States, the addition of lecithin or other emulsifying agents is limited to 1% by weight.

29.5.5 Tempering

Tempering is the controlled cooling of melted chocolate with agitation to promote the formation of small stable fat crystals throughout the chocolate. Besides agitation, time and temperature are also critical factors in the tempering process.

Cocoa butter is a polymorphic fat in which the crystals have different characteristics, melting points, and stability. There are four major types, γ, α, β', and β, with β being the only stable form. The unstable forms will eventually recrystallize into the stable β form (Minifie 1989b; Kattenberg 2001) (Table 29.4). Stable cocoa butter crystals will provide the following desired properties (Hofberger 2001):

- Snap,
- Good gloss,
- Proper texture,
- Bloom resistance,
- Contraction for demolding, and
- Less permeable barrier (increased shelf-life).

TABLE 29.4 Polymorphic Forms of Cocoa Butter.

Form	Crystallization Temperature	Melting Range	Approximate Life
Alpha α	From gamma	70–75°F (21–24°C)	1 h
Beta β	72–92°F (22–33°C)	68–95°F (20–35°C)	Stable
Beta prime β'	32–72°F (0–22°C)	59–85°F (15–30°C)	1 month
Gamma γ	<63°F (<17°C)	Up to 63°F (17°C)	Very unstable

Source: Adapted from Cook (1982).

The cocoa butter used in chocolate manufacture will affect the physical properties of the chocolate. The origin of the bean will also affect its quality. Attributes affected include hardness, texture, mouthfeel, and melting point of the butter.

Tempering can be conducted manually or in an automatic tempering unit to form stable fat "seed" crystals. There are many types of units available using various methods to achieve the goal. In general, chocolate tempering involves heating the chocolate to approximately 110–115°F (43–46°C) to melt the fat crystals, followed by cooling with agitation to between 80 and 84°F (27–29°C), and subsequently reheating to about 86–88°F (30–31°C) before molding or coating. In general, dark chocolates are tempered about 1–2°F (0.5–1.0°C) higher than milk chocolate (Hofberger 2001). However, exact temperatures and procedures will depend upon the tempering equipment and type of chocolate used.

The four most common types of automated temperers include the following.

- *Tempering kettle*. This is primarily a batch operation where the proper temperature is maintained by a jacketed water kettle, and agitation necessary for crystallization is provided by the sweeping action of the stirrer.
- *Plate heat exchanger*. The cooling and warming cycle is accomplished when chocolate passes over a series of jacketed plates. The scraping action of the plates allows rapid growth of small crystals. This is currently the most popular and common type of temperer.
- *Screw-type temperer*. As chocolate passes through a jacketed shell, the scraping action of the screw provides the agitation necessary for seed formation. Different sections of the shell provide the proper temperatures needed.
- *Bowl-type temperer*. This method uses dry heat to melt chocolate in a rotating bowl. Chocolate is melted to ~105°F (40–41°C), and then cooled by ambient air to approximately 86°F (30°C). During this process, tempered pieces of chocolate are placed in the back half of the divided bowl to provide the proper "seed" crystal. After the chocolate is sufficiently tempered, the temperature is raised to 88–90°F (31–32°C) to prevent overtempering. This method has the advantage of not requiring external water and plumbing. Room temperatures must be kept at 73°F (23°C) or less to provide for proper tempering. These units are gaining in popularity for very small candy shops where the use of "real" chocolate is desired.

The viscosity of the chocolate increases in proportion to the increase in seeding during tempering, thus chocolate must be used fairly quickly, or carefully heated to remelt some of the seed to prevent overtempering and achieve a steady state of temper. Overtempered chocolate may cause problems such as dull finish, excessive air bubbles, and poor mold release because of reduced contraction of the chocolate. Undertempered chocolate will also have poor mold release and will have a tendency to "finger print", and have premature blooming.

Once the desired pieces of chocolate are formed, the chocolate should be cooled gradually to prevent future problems. Initial cooling temperature should be approximately 65°F (18°C) with minimal air movement. This is necessary for the continued formation of stable crystals and to prevent case hardening or the formation of a "skin". Temperatures can gradually be decreased to about 45°F (7°C) with increasing air velocity. Final cooling temperature before packing should approach that of the room temperature (about 68°F) (20°C).

One must take into consideration the heat load of the product and adjust air temperatures and velocities accordingly when sending product through a cooling tunnel. Small pieces

of chocolate will set at a faster rate than large molded blocks. The temperature on the discharge end should be higher than the dew point to prevent condensation on the chocolate. At this point, the chocolate may appear solid. But, in fact, only about 70 to 75% of the cocoa butter is crystallized. It will take about 48 h for all of the cocoa butter to crystallize. So packing and storage conditions are still an important consideration (Hofberger 2001).

29.6 PRODUCTION METHODS

29.6.1 Enrobing

The mechanized form of hand dipping is enrobing. By completely covering the center with chocolate, the shelf-life of the product may be extended. This is primarily applicable to centers that, if not covered, could be prone to moisture loss, oxidation, or microbial action.

To ensure a firm bottom, manufacturers often first pass the centers through a prebottoming step. The prebottoming step can be done with chocolate or compound coatings. The advantage of using compound coatings is that they may be set up faster and no tempering is needed. The prebottoms need to be set before entering the enrober. This is especially important when lauric-based compound coatings are used, because they will most likely eventually bloom due to fat incompatibility.

Maintaining a consistent temper in the enrober will affect the coverage and appearance of the final product. Most enrobing units today utilize active tempering/detempering equipment.

Enrobing equipment with active tempering may have either inboard or outboard tempering. For smaller operations, an inboard unit that is part of the enrober provides for an inexpensive and compact unit. For larger operations, an outboard or separate tempering unit would be more suitable. It can also be fitted with a screen to filter inclusions coming from the center mass. Both methods follow the principle of enrobing centers with a tempered coating. The excess coating is removed, detempered, and retempered before entering the enrobing process again. This helps to prevent overseeding of the chocolate in the enrober. Chocolate temper should be taken at the enrober and not at the tempering machine, where line temperatures and residence times from the tempering machine to the enrober can significantly affect the amount of seeding.

It is important that the centers entering the enrober be maintained at 70–75°F (21–24°C), and the enrobing chocolate have the desired viscosity and rheological properties. Warmer centers may lead to possible bloom problems as the heat tries to escape from the interior of the enrobed product. Cold centers may also cause blooming and cracking of the coating shell due to expansion of the center mass as it warms.

The prebottomed centers are conveyed on a wire belt through a curtain of tempered chocolate, coating the top, sides, and bottom as well. The excess coating is removed through the action of adjustable vibratory shakers and forced air blowers. The blowers will often leave a desired rippled decoration on top. Prior to transferring to the conveyor belt, the product passes over a rotating detailer rod to remove the trailing "tail" at the end of the enrobed piece. For maximum shelf-life, it is important that the centers be completely covered, with no pinholes.

29.6.2 Cooling

After enrobing, the product enters a cooling tunnel to allow the coating to harden. To avoid blooming problems, temperature changes should be gradual, and the relative humidity

properly controlled. If the dew point is lower than room temperature, moisture could condense on the product and cause sugar bloom during storage.

There are three basic types of cooling tunnels that allow for the proper crystallization of the coating:

- Convection tunnels use chilled air moving in parallel under the product belt. The cold air enters near the discharge end of the tunnel, and moves counter current to product flow, and is removed near the entrance to the cooling tunnel.
- Multizone tunnels have chilled air moving transversely to product flow in specific sections. These types of coolers allow precise temperature control and even have the capability of re-warming the product prior to discharge to prevent condensation.
- Radiant cooling tunnels utilize black matte absorption plates. They absorb the radiant heat from the chocolate that supposedly helps to prevent case hardening of the chocolate. To increase the cooling rate, many radiant tunnels also use convection air movement.

29.6.3 Depositing and Molding

Deposited and molded chocolates are popular with consumers. For molded chocolates, design detail and uniformity can be controlled to a greater extent than with enrobed chocolates. For optimal quality and throughput, it is especially important that environmental conditions and the chocolate itself be closely monitored. Environmental factors include room temperature and relative humidity, and important chocolate issues include degree of temper, temperature, rheology of the coating, and time.

Different operations will require different temper, viscosity, and rheology of the chocolate. Improperly tempered chocolate will not have the proper contraction, set-up time, gloss, and resistance to bloom. This especially affects molded chocolates in that proper viscosity and temper are crucial for the proper filling of the molds as well as the demolding operation.

29.6.3.1 Depositing. Also known as drop depositing, this operation produces the familiar chocolate chips, caps, and stars. Tempered chocolate is deposited through a multi-piston block, through a depositor plate and nozzle, and onto a moving belt.

While the chocolate is still soft, nonpareil items may be made by covering the product with nonpareil seeds. As the coating hardens in the cooling tunnel, some of the seeds adhere to the surface, with the excess seeds separated out after exiting the tunnel.

Chocolate with inclusions such as nuts and crisp rice can also be deposited onto a moving belt. Most inclusions are mixed with the chocolate before it reaches the depositor. This can be done in a batch or continuous process. One needs to consider the density of the inclusion in relation to the chocolate because less dense inclusions tend to float and denser inclusions will have a tendency to sink.

The coating should have a high viscosity to give it good stand-up qualities and inhibit flow and distortion after depositing.

29.6.3.2 Molding. The molding process can be either done manually or mechanically. The main types of molds include solid, shell, and hollow molding.

Solid Molding. Of all the types, solid molding has been around the longest and is used primarily to produce small novelty items and bars to the 10-lb (4.5-kg) blocks commonly used in the industry. Tempered chocolate by itself, or mixed with inclusions such as nuts, raisins,

crisp rice, and so on, is deposited into traditional metal or the more popular polycarbonate molds that are the reverse image of the desired end product. The molds are shaken to evenly distribute the chocolate and remove air pockets. If necessary, the mold may be scraped to remove excess chocolate before entering the cooling area. After cooling, the hardened chocolate is inverted and the mold twisted or tapped to release the product. The molds are then warmed to the appropriate temperature before returning to the depositor. This is necessary to prevent blooming of the bars and for proper set-up of the chocolate.

For solid molding, a chocolate with a low plastic viscosity (PV) is desired for the proper release of entrapped air. If inclusions are added, a medium-viscosity chocolate should keep the particulates in proper suspension.

Shell Molding. There are several types of shell molding; however, all are a fairly complex operation where a center is enclosed in a chocolate shell. The centers are usually somewhat soft, from truffles, fudges, creams, and cordial cherries to semiliquid caramels and liqueurs.

For the classic shell process, tempered chocolate is deposited in molds, vibrated to remove entrapped air, then inverted and shaken to remove most of the chocolate, leaving a thin shell. The chocolate is cooled and the filling is deposited. The mold surface and shell rim are briefly warmed before chocolate is deposited on the filling. This allows for the complete adhesion between the shell rim and the bottom coat of chocolate. The excess chocolate is scraped off and the mold is cooled, allowing the candy to fully contract. After sufficient cooling, the product is demolded and packaged.

Recent developments in shell molding equipment allow for the chocolate and filling to be produced in one depositing sequence that is commonly called a one-shot deposit. It is possible to deposit a warm homogeneous center mass such as creme fondant, caramels, truffles, and meltaways. In this process, two separate hoppers, one with chocolate and the other with the filling are deposited through concentric nozzles with precision timing to insure that the center is surrounded by chocolate. The mold is then vibrated, cooled and demolded.

Hollow Molding. With hollow molds, a mold is partially filled with chocolate, then closed and rotated on a spinner to distribute the chocolate throughout the mold. The items most commonly associated with hollow molding include novelty items such as Easter eggs, bunnies, and Santa Clauses. Hollow molding is still primarily done in batches on a spinning machine that rotates 360° through all axes. Chocolate is deposited in a two-piece mold that is then closed (with clips, hinges, or magnets) and spun. After the chocolate has cooled to the point where it no longer flows, the molds are cooled and then demolded. Chocolate used for this operation should have a low viscosity and yield value to provide a thin shell.

29.7 CONFECTIONERY COATINGS

In the United States, chocolate must conform to a certain Standard of Identity. If it does not meet the specifications, it cannot legally be called chocolate. The FDA has established Standard of Identities for cocoa products and other commonly used cocoa-based products (Table 29.1).

29.7.1 Compound Coatings (Vegetable Fat Coatings)

These are similar to real-chocolate coatings except that chocolate liquor and cocoa butter have been replaced with cocoa powder and less expensive vegetable fat, respectively. The

ingredients are dry mixed and refined to a smooth texture, with conching kept to a minimum. White and pastel compound coatings consist of vegetable fat, sugar, and dairy powder, with added flavors and colors.

The vegetable fats substituted for cocoa butter may be classified as lauric and nonlauric fats. Lauric fats are short-chained fatty acid glycerides that have physical properties similar to cocoa butter but are incompatible with cocoa butter. Examples are fractionated coconut and palm kernel oils. They have good stability, texture, and flavor release. Tempering is not needed and it is less expensive than cocoa butter. However, when exposed to lipases, especially in the presence of moisture, the free fatty acids released will have a strong soapy taste (Minifie 1989c).

The nonlauric fats are composed of longer-chain fatty acids and include hydrogenated soy, palm, and cottonseed oils. Unlike lauric fats, the nonlauric fats can be blended with up to 25% cocoa butter and chocolate liquor for a stronger flavor. Due to texture differences, the nonlaurics are used primarily for covering baked goods where a less brittle (or snap) texture is desired. Nonlauric fats are also susceptible to decomposition by lipases; however, the fatty acids liberated do not have a soapy flavor (Minifie 1989c).

29.7.2 Sugar-Free Coatings

Sugar-free coatings have been available to the food industry for over 30 years. The original versions were difficult to manufacture. With the advent of technology and health issues, the quality and quantity of sugar-free coatings and their related products have increased tremendously.

According to the FDA, a sugar-free food contains less than 0.5 g of sugars per reference serving size (US Code of Federal Regulations 2004). For most confections, the reference serving size is 40 g, so the 0.5 g/40 g equates to less than 1.25% allowed sugars. Common examples of sugars are glucose, sucrose, fructose, and lactose. Confections that are "no sugar added" generally have no added sucrose, but may contain another sugar such as lactose, which is found in milk powder.

At the present time there is no Standard of Identity for sugar-free chocolates in the U.S. Code of Federal Regulations. From a legal standpoint, this product cannot be called "chocolate". To minimize the sugar level, caseinates are often substituted for the milk as milk contains sugars in the form of lactose.

The milk powder generally used for traditional chocolates contains naturally present lactose over 50% of the nonfat dried milk. This does not meet the requirements of a sugar-free label and can only be used for "no sugar added" coatings. Sodium caseinate and to a lesser extent calcium caseinate are most commonly used as a milk replacer for sugar-free coating. Sodium caseinate at a 4–9% level will be sufficient to replace a traditional milk chocolate containing 12–20% milk solids (Hofberger 2003).

Sugar alcohols as a group have always been considered the key to making a good sugar-free coating. With the exception of xylitol and in some instances maltitol, they are often combined with high-intensity sweeteners such as aspartame, acesulfame K, and sucralose to achieve the same sweetness level as sugar-based coatings. Aspartame is more sensitive to heat and acidic conditions. Acesulfame K in high concentration can give a bitter aftertaste. Aspartame has the disadvantage of having to require a "contains phenylalanine" warning label when used (Hofberger 2003).

Most formulations using a high proportion of sugar alcohols will require a label statement of "excess consumption may have a laxative effect." If a sugar-free claim is made, it

must also include a "not a reduced calorie food" statement unless the caloric value is at least 25% less than the standard product.

29.8 CHOCOLATE BLOOM PROBLEMS

The most common problem encountered during chocolate storage is probably blooming. It is a defect that manifests itself as a grayish-white film on the surface of the product. In most instances the eating quality is not affected; however, the appearance is not very appetizing. Bloom may occur anywhere from a few hours after production to several months later (Seguine 2001). There are basically two types of bloom – sugar and fat.

29.8.1 Sugar Bloom

Sugar bloom occurs less frequently than fat bloom and its appearance may look like fat bloom. It occurs when the surface of the chocolate is exposed to moisture or high humidity and then dries out. The surface film of water dissolves some of the sugar particles in the chocolate, which then recrystallizes upon drying into a dull grayish-white haze. It commonly occurs in chocolate products emerging from a cooling tunnel into a warm and humid room. It also may occur when product is brought out of cold conditions and subjected to warmer, humid conditions without proper packaging and time to gradually bring it up to room temperature. In general, sugar bloom feels dry to the touch and does not melt, which differs from fat bloom.

29.8.2 Fat Bloom

Fat bloom is the visible accumulation of large cocoa butter crystals on the chocolate surface, which may give it a greasy surface texture that melts readily when touched. It may be accompanied by numerous mini cracks that give it a dull grayish-white appearance. These changes are primarily due to the polymorphic nature of cocoa butter and the migration of liquid fat (McCarthy and others 2003). Some factors causing chocolate bloom are listed in Table 29.5.

TABLE 29.5 Some Factors Causing Chocolate Blooming.

- Cold molds, warm to 75–80°F (24–27°C)
- Cooling tunnels/rooms that are extreme cold
- Extreme water tempertaure used in cooling/heating
- Improper formulation of centers that allow moisture or oils to migrate to the outer surface of the coating
- Introduction of foreign fats (such as coconut oil) into the chocolate
- Packaging/shrink-wrap of finished goods with heat can cause localized bloom
- Placing chocolates in cold storage before fully solidified; solidification takes 24–48 h
- Processing line equipment heaters that detemper chocolate
- Storage temperature exceeding 88–90°F (31–32°C); ideally $<$68°F ($<$20°C)
- Temperature fluctuations during storage
- Too cold or hot center mass when enrobing; ideally 70–75°F (21–24°C)
- Undertempered chocolate

Source: Adapted from Hofberger (2001).

29.9 STORAGE AND HANDLING

Like many other foods, chocolate flavor will change over time, resulting in a more balanced flavor profile. In general, most chocolates should be contained in a nonpermeable barrier for protection from oxygen, moisture, and light. Ideally, chocolate should be stored at 65–68°F (18–20°C) and less than 50% relative humidity. Dark chocolate is less prone to moisture absorption during storage than milk chocolate (Minifie 1989g; Hofberger 1999b; Urbanski 2001). As it is fat-based, it will readily absorb off-odors so chocolate should be stored in an odor-free environment. Chocolate will also pick up odors from packaging materials, printing inks, as well as odors generated from the heat sealing or shrink-wrapping process. Advances in packaging technology now include water-based cold-seal adhesives that not only eliminate the potential for heat-damaged chocolates but are also low in odor, thus eliminating or reducing the possibility for the development of off-flavors or smells (Barry 2003).

Properly stored, solid chocolate should have a shelf-life of one year of more. Chocolate can be frozen for even longer storage; however, care must be taken when bringing it back to room temperature from the frozen state to avoid condensation.

If chocolate is manufactured from clean beans under sanitary conditions, its microbial count should be low due to its low water activity (a_w) level. However, chocolates may become contaminated by some of the ingredients and equipment used, and improper storage conditions.

Moisture can be introduced into the product during its manufacture (such as equipment leaks or improperly dried equipment) or during storage under damp conditions. This may lead to mold problems. Equipment leaks will produce a high-viscosity chocolate that is readily apparent and should not be used for further processing. Moisture on the outer surface of the chocolate from improper storage conditions can also cause mold growth.

Rancidity is generally not a problem in milk and dark chocolates because of the natural antioxidant properties of undeodorized cocoa butter. Rancidity can be found on occasion in white chocolate and compound coatings and is catalyzed in the presence of air, moisture, light, heat, and some metals. With oxidative rancidity, the fats or oils exposed to air produce objectionable flavors due to the formation of aldehydes and ketones. The addition of synthetic antioxidants will help inhibit this reaction although some fats contain natural antioxidants as well (Minifie 1989g).

Hydrolytic rancidity, also known as soapy rancidity, is due to the lipid hydrolysis of fat into glycerol and fatty acids with the production of an off-flavored soapy taste. Lipases may be present in ingredients such as coconut, milk products, egg albumen, and cocoa. However, not all lipase activity will result in a soapy flavor. If possible, it is recommended that ingredients susceptible to hydrolytic rancidity be heat treated to inactivate the enzyme. Fats containing lauric acid, even a trace amount, will yield a soapy flavor. Coconut oil and palm kernel oil contain 40–50% lauric glycerides, and butter fat contains 2–6%. Cocoa butter and palm oil do not contain lauric acid (Minifie 1989g).

29.10 NUTRITIONAL VALUE

Chocolate is not a major source of dietary fat, but it does contain fat. The majority of the fat comes from cocoa butter and is composed mainly of three fatty acids: 30–37% of the monounsaturated fatty acid is oleic acid (18 : 1), 32–37% of the saturated fatty acid is stearic acid (18 : 0), and 23–30% of saturated fatty acid is palmitic acid (16 : 0). It also

TABLE 29.6 USDA Nutrient Database for Standard Reference, Release 15 (July 2002)[a].

Item	kcal	Protein (g)	Fat (g)	Carbohydrate (g)	Calcium (mg)	Saturated Fat (g)	Monounsaturated Fat (g)	Polyunsaturated Fat (g)	Cholesterol (mg)	Caffeine (mg)	Theobromine (mg)
Semisweet chocolate	136	1.2	8.5	17.9	9.1	5.0	2.8	0.3	0	17.6	137.8
Sweet chocolate	143	1.1	9.7	16.9	6.8	5.7	3.2	0.3	0	18.7	120.8
Unsweetened chocolate	145	2.9	15.7	8.0	21.0	9.1	5.2	0.5	0	57.1	346.4
Milk chocolate	145	2.0	8.7	16.8	54.1	5.2	2.8	0.3	6.2	7.4	47.9
Cocoa butter	251	0	28.4	0	0	16.9	9.3	0.9	0	NA	NA
Cocoa powder	63	5.1	3.7	15.5	31.5	2.2	1.2	0.1	0	22.1	746.7
Brewed coffee, 8 oz	5	0.2	0	0.9	4.7	0	0	0	0	137.5	NA
Instant coffee, prep. 8 oz	5	0.2	0	0.9	7.1	0	0	0	0	76.4	NA
Brewed coffee decaff, 8 oz	5	0.2	0	0.9	74.7	0	0	0	0	2.4	NA

Source: USDA Nutrient Database for Standard Reference (2002).

[a]Values are per 1 oz (28.35 g) unless otherwise noted.

contains 2–4% linoleic acid (Nacional de Chocolates 2003). Although stearic acid is classified as a saturated fat, it does not appear to raise cholesterol levels the way other saturated fatty acids do (Chocolate Information Center 2003a). Table 29.6 shows the USDA nutrient content of several types of chocolate.

Chocolate has also been noted recently to contain relatively high amounts of polyphenols, in particular the flavanols, catechin, and epicatechin. Polyphenols are found in the plant kingdom and exhibit antioxidant activity that is thought to reduce the risk of some human diseases. The levels found in chocolate and related products will depend upon cocoa bean variety, fermentation, and subsequent processing and formulation (Chocolate Information Center 2003b; International Cocoa Organization 2003a).

A class of alkaloid molecules known as methylxanthines (such as caffiene, theobromine, and theophylline) occurs naturally in a number of plant species. They exhibit similar pharmacological properties. They are mild stimulants and possess mild diuretic properties (About Chemistry 2001; International Cocoa Organization 2003b). A common belief is that chocolate contains a lot of caffeine. However, caffeine is the primary alkaloid in coffee, theobromine the primary alkaloid in chocolate, and theophylline the primary alkaloid in tea. A comparison of the caffeine contents in chocolate and coffee is presented (Table 29.6).

A food allergy is a reaction by the body's immune system to a substance, usually a protein, in the food. According to the National Institutes of Health, approximately seven million Americans (more children than adults) have a true food allergy. Eight food allergens account for 90% of all allergic reactions. These include milk, eggs, peanuts, tree nuts, soy, wheat, fish, and shellfish (Chocolate Manufacturers Association 2003b; National Confectioners Association 2003). An allergy to chocolate itself is uncommon; however, the addition of other ingredients may cause an allergic reaction.

Chocolate is one of the most popular flavors, enjoyed by millions of people in a variety of ways, from beverages, to main dishes, sauces, baked goods, and of course confections. Eaten in moderation it can be part of a healthful diet.

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30

Confectionery: Inspection and Enforcement

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30.1 Establishment Inspection for Chocolate and Cocoa Products 696
30.1.1 Raw Materials 696
30.1.2 Processing 697
30.1.3 Sample Collection 697
30.1.3.1 In-Process Sampling 697
30.1.3.2 Finished Product Sampling 697
30.2 Establishment Inspection for Candy Without Chocolate, Candy Specialties, and Chewing Gum 698
30.2.1 Raw Materials 698
30.2.2 Equipment and Manufacturing Processes 698
30.2.2.1 Molding Starch and Trays 698
30.2.2.2 Scrap Candy (Rework) 698
30.2.2.3 Food and Color Additives 698
30.2.3 Sample Collection 699
30.2.3.1 In-Process Sampling 699
30.2.3.2 Finished Product Sampling 699
30.3 Enforcement Activities for Candy and Sweeteners 699
30.3.1 Boxed Candies Recalled due to Undeclared Egg Whites and Yellow #6 700
30.3.2 Imported Candy Seized 700
30.3.3 Konjac Candy 700
30.3.4 Seizure of Honey with Chloramphenicol 701

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30.1 ESTABLISHMENT INSPECTION FOR CHOCOLATE AND COCOA PRODUCTS

All information in this chapter has been modified from several public documents available at the website of U.S. Food and Drug Administration (FDA). The discussion resembles the format of teacher-student. The supervisor is describing the procedures to the inspection personnel. The objective of the presentation in this chapter is to assist the plant operation manager of a food company to become familiar with a food processing establishment inspection routinely conducted by the federal agency, FDA.

Food plant inspections are conducted to evaluate the methods, facilities, and controls used in manufacturing, storage and distribution of chocolate and chocolate products foods. Before an inspection, review FDA's guides on the general procedures. Direct special attention to the following areas when inspecting establishments processing chocolate and chocolate products.

30.1.1 Raw Materials

Insects that commonly infest cocoa beans are stored product insects representing an avoidable defect. Cocoa beans are often fumigated to kill these insect pests and cocoa bean processing effectively removes any such filth where the insects have not invaded the interior or nib portion of the bean. During inspections, determine that effective measures such as physical barriers are in place to prevent cross contamination between cocoa bean cleaning and finished product processing operations.
If cocoa beans are received in bags proceed as follows:

1. Screen a number of lots in storage by conducting a preliminary field examination. Field examine a representative number of bags in the lot. Be alert for live stored food insect pests on or around the bags including adult and larval beetles and moths. Document findings with photographs and collect samples as instructed by FDA guidelines. Extract approximately one pound of beans from each bag field examined utilizing an appropriate sized trier. Screen the beans on a number 3 sieve. Examine siftings for the presence of insects, rodent excreta and other extraneous material.
2. Composite one-hundred cocoa beans from ten to twenty bags in the lot.
3. Crack open each bean and break the interior portion (nib) of the beans into small pieces along the natural folds of the nib material. This can be accomplished with a hammer, pruning shears or pliers. Examine for mold and/or insect damage. A moldy bean is defined as any bean showing extensive mold effecting a quarter or more of the exposed nib material. Insect damaged is defined as any bean showing insects (fragments or whole insects), insect excreta, webbing or tunneling.
4. Collect an official sample for laboratory examination if field exam reveals:
   a. 2% or more moldy beans; or
   b. 2% or more insect damaged beans; or
   c. 3% or more moldy plus insect damaged beans.

If cocoa beans are received un-bagged in cargo containers obtain the subsample from random locations and levels within the container utilizing a grain probe.

If examination of raw material beans indicates the presence of internal insect damage and/or mold, document with appropriate samples, the use of those beans in the manufacture of processed chocolate products.

Determine if critical raw materials including non-fat dry milk (NFDM) and chocolate are received under a Salmonella Free Certificate.

Evaluate the firm's testing of raw materials for bacterial load, including Salmonella and other pathogens.

30.1.2 Processing

Determine in detail the firm's cocoa bean processing to include the following.

1. Precleaning: Use of magnets to remove metal.
2. Blending: Determine percent of different beans in blend.
3. Roasting: Time temperature relationship. Determine if ovens have recording thermometers.
4. Cooling: Time and to what temperature.
5. Cracking and fanning operations.
6. Milling.
7. Storage of chocolate liquor: temperature of storage tanks.

Be especially alert for avenues of contamination by water or condensation. The presence of moisture can result in conditions that can support the growth of Salmonella.

Ascertain firm's coding system.

30.1.3 Sample Collection

30.1.3.1 In-Process Sampling

1. Collect in-line samples and exhibits to document insanitary conditions which may result in contamination of the finished product with filth, mold, bacteria or other factors.
2. When the sample is collected for Salmonella and other bacterial examination, include 30-100 gram (4 oz) subs of the finished product.

30.1.3.2 Finished Product Sampling

Filth: Cocoa Beans. Take the square root of the number of bags in the lot to determine the number of subs to collect. Multiply by three to determine the number of bags to sample. Each sub will consist of 450 grams (1l b) of beans composited by collecting approximately 170 grams (1/3 lb) from each of three bags. You can sample up to a maximum of seventy-five bags which would yield twenty-five subs but in no case collect less than six subs. Fore more details, consult FDA's guidelines.

Filth: Cocoa Powder, Press Cake, Expeller. Cake, Chocolate. For microscopic filth, excess shell, etc., sample the square root of the number of bags in the lot. Collect a minimum of six and a maximum of eighteen subs each consisting of 900 grams (2 lbs) taken 340 grams (2/3 lb) from each of the three bags. Collect the subs in duplicate for

the 702(b) portion. For retail size containers, sample the square root of the number of containers in the lot with a minimum of six and a maximum of 18–900 gram (2 lb) subs.

Consult FDA guidelines for microbiological and standard sampling.

30.2 ESTABLISHMENT INSPECTION FOR CANDY WITHOUT CHOCOLATE, CANDY SPECIALTIES, AND CHEWING GUM

For these types of manufacturing establishments inspection, direct special attention to the following points.

30.2.1 Raw Materials

Egg and milk products must be pasteurized before use or otherwise treated during processing to destroy viable Salmonella microorganisms. Steps must also be taken to ensure that other raw materials susceptible to contamination by pathogenic microorganisms (i.e., gelatin, dried coconut, nuts) are free of such organisms before use unless they are pasteurized or otherwise treated before or during processing. Materials capable of supporting growth of pathogenic microorganisms must be held at appropriate temperature and relative humidity except for the period of time actually required for processing and not so long as to effect the wholesomeness of the product. Raw materials such as nuts and corn meal that are susceptible to aflatoxin contamination must comply with FDA regulations, guidelines, and action levels for poisonous or deleterious substances or be received under the supplier's guarantee of such compliance.

30.2.2 Equipment and Manufacturing Processes

Evaluate the control of foreign objects embedded or hidden within candy such as wooden splinters, glass, or otherwise dangerous articles. Processors must take every reasonable precaution to ensure that production procedures do not contribute contamination from any source. For example, strategically located magnets and/or metal detectors may be necessary to prevent contamination by metal.

30.2.2.1 Molding Starch and Trays Molding starch is often contaminated. Determine how long it has been in use. Estimate amounts and frequency that fresh starch is added. In addition, if wooden starch trays are utilized, check for splitting and/or splinters.

30.2.2.2 Scrap Candy (Rework) Evaluate the handling and reworking of scrap candy. Determine if containers used for rework can be readily distinguished from containers used for waste. Determine the source of scrap candy for rework.

30.2.2.3 Food and Color Additives Identify the slab dressing used, and estimate the amount per batch. Slab dressings are lubricants employed as release agents on such equipment as cooling tables used in the manufacture of hard candy. If mineral oil is used at a level higher than 2000 ppm (0.2%, approximately 100 g (3½ oz) per 91 kg (200 lb) batch), obtain shipments for finished product sampling.

Regulation limits the amount of alcohol that confectionery may contain to one-half of 1% by volume, and this must be derived solely from the use of flavoring extracts. However, this does not apply to confectionery designed for interstate commerce, if the sale of such confectionery is permitted under the laws of the state in which such confectionery is intended to be offered for sale.

30.2.3 Sample Collection

30.2.3.1 In-Process Sampling

Molding Starch. Collect the following subsamples when insects or rodent excreta are observed near molding starch operations:

1. One pint of starch from the starch mogul;
2. One pint of starch from starch trays in use;
3. One pint of tailings from the starch cleaner.

Scrap Candy (*Rework*). When appropriate collect exhibits and/or official samples of contaminated scrap, and describe how it is used. If contamination by filth is suspected, collect representative exhibits and/or official samples of scrap, even though no macroscopic filth is evident.

Deleterious Articles. Collect samples of products (usually 3–6 units) that have sharp pointed articles or otherwise dangerous articles embedded or hidden within candy (e.g., wooden sticks, pins, trinkets) when a potential for injury exists.

30.2.3.2 Finished Product Sampling

Mislabeled Chocolate Products. The current standards for cocoa products can be found in 21 CFR Part 163. When mislabeling is caused by a reference to chocolate, documentation should include a reference sample of the ingredient being substituted and this ingredient's quantitative formula and labeling. Be aware that FDA may issue temporary permits for test marketing of products that deviate from the standards.

Deceptive Packages. Submit specimens of flagrantly deceptive packages consisting of three filled and three empty packages with all "spacers" and fillers.

Refer to FDA guidelines for sampling filth and microbiological status.

30.3 ENFORCEMENT ACTIVITIES FOR CANDY AND SWEETENERS

The FDA rigorously enforces the law when violations are uncovered in a routine inspection. Usually, other warning signs alert the FDA to such violations. This section describe some such violations in confectionery products.

All information on these enforcement activities has been modified from public documents at the FDA's official government website.

30.3.1 Boxed Candies Recalled due to Undeclared Egg Whites and Yellow #6

FDA's Seattle District Office reported that on May 9, 2002, a chocolate company in Portland, Oregon, initiated a Class I recall of their CLASSIC 9 piece, CLASSIC 16 piece, and 12 Piece Spring Collection boxed candies due to undeclared egg whites and yellow #6. The boxed candies contained a toffee truffle with a flower decoration on top. The flower decoration contained the undeclared egg whites and yellow #6. The products were sold in retail stores and through mail order.

30.3.2 Imported Candy Seized

FDA's Southwest Import District (SWID) recommended the seizure of 192 cases of Hormigas candy that was found to be in whole or partly substituted for candy refused admission by FDA. The candy was manufactured by a company in Mexico and imported into the United States and refused admission by FDA due to filth contamination.

The U.S. Customs' special agents conducted actual seizure of the substituted merchandise. In addition to the seizure, U.S. Customs considered assessing liquidated damages against the importer for failure to redeliver the actual merchandise refused admission by FDA.

30.3.3 Konjac Candy

On October 5, 2001, FDA issued a second warning to the public concerning the serious choking hazard caused by konjac candy. FDA issued the first warning on August 17, 2001. Konjac candy is a fruit-flavored gel candies in various flavors that is imported primarily from Asia and has been linked to six deaths in children in the United States. FDA decided a second warning was warranted after consultation with experts on choking from the Consumer Product Safety Commission (CPSC). CPSC staff confirmed that these candies posed a serious choking risk, particularly to infants, children, and the elderly. FDA also issued an import alert to address the problem of importation of these candies from other countries. The agency worked with numerous firms in a nationwide recall of these candies. One firm refused to recall their candies and the product was seized by FDA following repeated attempts to get the firm to recall the candies.

FDA announced a recall by a Puerto Rico firm of 1500 cartons of Fruzel assorted Natural Fruit Jelly Candy because these products present a choking hazard. The product was distributed to wholesale and retail establishments throughout Puerto Rico under the "Neo USA" brand. The mini jelly candies came in assorted flavors. Each mini jelly cup is about the size of a single-serve coffee creamer. The candies were packaged in 16.5 g jars with 88 units per jar.

On May 17, 2002, FDA announced a recall by a firm in Bayonne, NJ, of 1197 cases of mini jelly candies (or mini cup gel candy) because these products presented a choking hazard. The product was distributed to retail establishments throughout New York, Massachusetts, Illinois, Florida, Georgia, Maryland, New Jersey, Pennsylvania, and Virginia under the "ABC" brand.

On April 25, 2002, FDA announced a recall by a company in Chicago, Illinois, of 3115 bags of mini jelly candies (or mini cup gel candy) because these products present a choking hazard. The product was distributed in bags which read in part; "Mi Costenita . . . Gelatinas Coconut Jelly" to retail establishments in Illinois, Michigan, Ohio, Indiana, Tennessee, Arkansas, Missouri, Mississippi, Kansas, Alabama, Georgia, North Carolina, and Wisconsin.

On April 25, 2002, FDA announced a recall by another company in Chicago, Illinois, of 464 cases of mini jelly candies (or mini cup gel candy) because these products present a choking hazard. The product was distributed to retail establishments throughout northern Illinois and Wisconsin under the "Jojomo" and "Naluwan" brand. The label described the product as "JM Jojomo" and "Naluwan Nata De Coco Konnyaku Jelly" in all flavors. The candy came in small creamer-sized sealed plastic cups.

On April 5, 2002, FDA announced recalls from a company in the City of Industry, California, and one in Vernon, California, of 100 cases of mini jelly candies (or mini cup gel candy) because these products present a choking hazard. The product was distributed to retail establishments throughout southern California under the "Sugarland" brand. The label described the product as "Jellyace Buko Pandan". The candy came in small sealed plastic cups.

On February 15, 2002, FDA reported a recall by an Asian company in Chicago, Illinois. These candies were distributed in Illinois, Iowa, Indiana, Kentucky, Ohio, Wisconsin, Florida, Michigan, Missouri, South Dakota, and Kansas. The three brand names included "Don Empire", another with no brand name and only Chinese characters and butterflies on the label, and "My Love and Coco". The individual serving cups were packaged in 300 g plastic bags with 30 bags per case, 510 g plastic panda-bear-shaped jars with 12 jars per case, and 1200 g round plastic jars with 6 jars per case.

There were numerous recalls of the same products sold under different brand names. Many of the recalled products were destroyed.

30.3.4 Seizure of Honey with Chloramphenicol

On August 28, 2002, FDA investigators from the New Orleans District accompanied the U.S. Marshals Service in a seizure of imported bulk, in-process, and finished-product honey located at Davidsonville, Louisiana. The product was valued at approximately $50,000,00. A sample of the imported bulk honey, collected by the New Orleans District, was tested by FDA's Denver District Laboratory and found to contain chloramphenicol at or above the level of 1 part per billion (ppb). The Louisiana Department of Agriculture and Forestry also conducted tests on samples of the imported honey and found the product contained chloramphenicol.

Chloramphenicol is a broad-spectrum antibiotic approved for use in humans with serious infections when other, less toxic, drugs are ineffective. It has not been possible to identify a safe level of human exposure to chloramphenicol due to the unpredictable dose–response on different patients. The main toxic effect of chloramphenicol in humans is the development of a type of bone marrow depression (aplastic anemia) in susceptible individuals. This condition is usually irreversible and fatal. In addition, the onset of the condition is not dose–dependent.

Prior to seizure, FDA had issued a letter to the U.S. Attorney on August 27, 2002, requesting that a Complaint for Forfeiture be filed in the U.S. District Court. The Complaint charged that the products were adulterated because they contained a food additive, namely chloramphenicol. The violations are that the products were misbranded because the label bears the statement "no additives", which is false and misleading in that the article contains the food additive chloramphenicol. There are health consequences that may result from the consumption of the article.

Section IX

Fats and Oils

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31

Margarine and Dairy Spreads: Processing and Technology

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31.1 Introduction 705
31.2 Ingredients for Margarine and Dairy Spreads 707
31.2.1 Fat Phase 707
31.2.1.1 Interchangeability of Fats and Oils 707
31.2.1.2 Other Fat Phase Ingredients 708
31.2.2 Water Phase 709
31.3 Crystallization Technology 709
31.4 Margarine Production 711
31.5 Industrial Products 716
31.6 Consumer Margarine, Spreads, and Dairy Spreads 718
31.7 Processing and Product Trends 722
References 722

31.1 INTRODUCTION

Margarine was invented and patented by the French chemist, Hippolyte Mege Mouries, in 1868 as a substitute to butter. A shortage of butter due to an increasing urban population and growing army prompted this invention. The margarine was traditionally based on animal fats and the process was a relatively simple mixing process that included simultaneous cooling (Applewhite 1985; Hoffmann 1989).

Over the years the original product range has multiplied into a large variety of fat products, and since 1940 it has been possible to produce margarine in closed continuous

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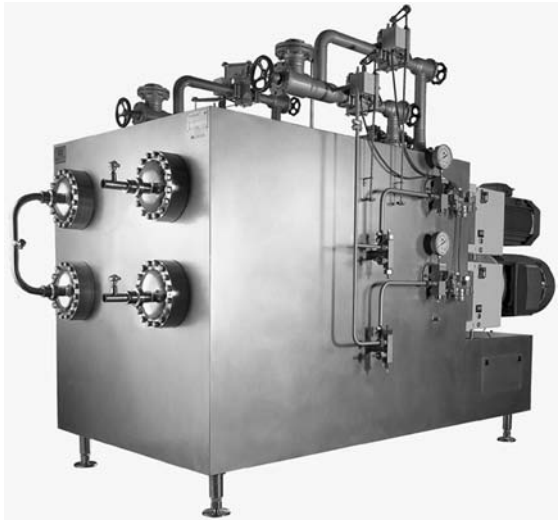

Figure 31.1 *Scraped surface heat exchanger type (1 + 1 + 1 + 1) × 250 Perfector (manufactured by Gerstenberg and Agger A/S).*

systems using scraped surface heat exchanger (SSHE) technology. Previously, margarine was mainly produced in open systems using a chilling drum. Today, both crystallization methods are still used in the industry: SSHE and drum chilling (Figs 31.1 and 31.2).

In today's market there are a range of products containing different types and amounts of fats and oils. Vegetable fats and oils are used to a great extent. After 1910 when hydrogenation came into practice in Europe, a greater variety of fats and oils was available and significant advances in the manufacturing process were made (Chrysam 1996).

The SSHE plant is by far the most flexible of the crystallization technologies in terms of crystallization of different types of fat products. The SSHE plant is designed to produce a large variety of fat products including consumer and industrial margarine, shortening, ghee, vanaspati (i.e., vegetable ghee), recombined butter, and dairy blends.

Consumer margarine includes all-purpose table margarine, soft table, reduced and low-fat spreads, and speciality products. Speciality products can be margarine containing flavours, spices, or functional ingredients.

Industrial margarine includes bakery products such as puff pastry margarine, cake and cream margarine, frying margarine, and bakery improvers.

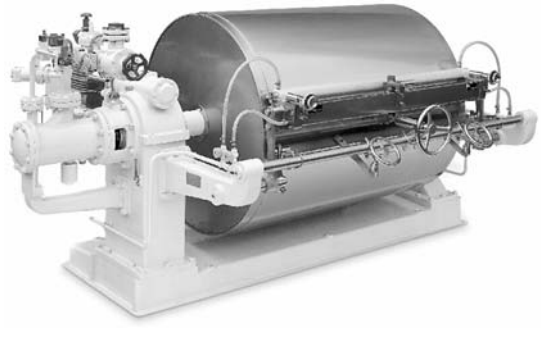

Figure 31.2 *Chilling drum type Diacooler (manufactured by Gerstenberg and Agger A/S).*

In this chapter, the crystallization of fats and oils used for margarine and spreads production along with the processing techniques and market trends will be described.

31.2 INGREDIENTS FOR MARGARINE AND DAIRY SPREADS

Margarine and related products contain a water phase and a fat phase. Depending on the nature of the product the recipe will typically consist of a fat blend, emulsifier(s), flavour(s), colour(s), water, salt, sugar, and preservatives. Stabilizers are often added to fat spreads.

31.2.1 Fat Phase

31.2.1.1 Interchangeability of Fats and Oils. Margarine, like butter, can be characterized as a water-in-oil (w/o) emulsion in which the water phase is finely dispersed as droplets in the continuous fat phase. The fat crystals form a three-dimensional network resulting in products with properties of plastic semisolid nature (Stern and Cmolic 1976; DeMan and Beers 1987; Juriaansee and Heerttje 1988). The major ingredients in the fat phase, the fat blend, consist normally of a blend of different fats and oils. In order to achieve margarine with the desired characteristics and functionalities, the ratio of fats and oils in the fat blend is very important. The objective is to obtain a defined solid fat content (SFC) measured by NMR at various temperatures, typically ranging from 5 to 40°C (NMR). The SFC profile (Fig. 31.3) describes the amount of solid phase compared to the liquid phase at the given temperature. The SFC profile will vary according to the type of product; cup-filled margarine such as soft table margarine contains the least solids at a given temperature and puff pastry contains the highest amounts of solids (Haighton 1976; Berger 1989).

Variations might occur within each product category, because margarine manufacturers around the world have different product specifications according to application. In addition, seasonal changes might force the manufacturers to modify the oil blend formulation several times a year, shifting to harder formulations from winter to summer.

The ingredients used in the formulation of the fat blend can be derived from any animal, vegetable, or marine oil source. The choice of fats will depend on legislation, price, quality, functionality, and market demands. However, the availability of fats can be limited due to legislation, religious prohibitions, or trade barriers (Berger 1989).

As shown in Table 31.1, it is possible to divide ingredients into three main categories: liquid oils, semisolid fats, and hard stocks. Liquid source oils are today considered to be completely interchangeable as components of margarine and shortening blends due to the modern refining processes. Fat-modification processes such as fractionation,

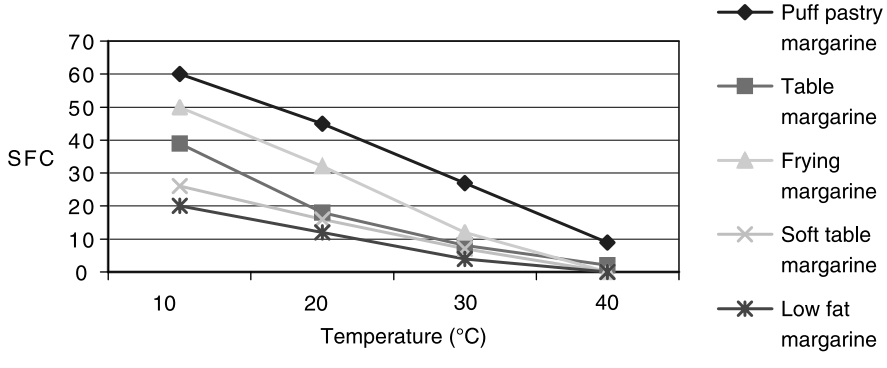

Figure 31.3 *SFC profile of selected margarine products (Gerstenberg and Agger 2001).*

TABLE 31.1 Selected Oils and Fats Grouped According to Melting Points (Berger 1989).

Liquid Oils	Semisolid Fats	Hard Stock
Unsaturated vegetable oils	Hydrogenated oils (MP: 32–34°C)	Hydrogenated oils and fats (MP > 40°C)
Palm olein	Butter fat (MP: ~34°C)	Interesterified oils and fats (MP > 40°C)
Palm kernel	Palm	Beef tallow
Coconut	Lard	Palm stearin

hydrogenation, and interesterification enable a high degree of interchangeability among fats. The modification processes produce, individually or in combination, the full range of fatty intermediates used in the manufacture of all types of margarines, and these processes enable fats to become almost completely interchangeable.

A particular product specification can be met by a large number of alternative formulations. A suitable blend can be made by combining one or more ingredients from each of the three groups (Berger 1989). By mixing the ingredients from the three groups in specific ratios, a suitable SFC profile can be met. If, for example, hydrogenated soy bean oil is used in soft table margarine and hydrogenated rapeseed is cheaper, the latter can substitute for hydrogenated soybean oil in the formulation. Thus, the producer attains the flexibility to interchange the best available and cheapest raw materials in an ever-changing market situation. The consumers are thereby certain to buy continuously a product with the same quality and price. However, certain technical limitations exist when combining one or more ingredients from the three groups. The crystallization habit or polymorphism of the fat can set limits on the proportion of the particular fat used in the blend. In addition, the crystallization rate of the fat blend is of great importance in regard to the configuration of the processing line.

31.2.1.2 Other Fat Phase Ingredients. Apart from the fat blend, the fat phase typically consists of minor ingredients such as emulsifier, lecithin, flavour, color, and antioxidants. These minor ingredients are dissolved in the fat blend before emulsification as described above.

Emulsifiers are surface-active compounds used to reduce the interfacial tension between the water and the fat phase. The emulsifier stabilizes the liquid emulsion before crystallization to secure a homogeneous product and provide fine and stable water distribution in margarine and spreads. The microbiological keeping properties in the final product are thereby improved. The most commonly used emulsifiers in margarine are distilled monoglycerides or mixtures of mono- and diglycerides. The best water-binding effect in margarine is achieved by using saturated monoglycerides (Danisco 2002c).

Soya lecithin can be used with the emulsifier to improve the effects of the emulsifier. To achieve the maximum effect of the emulsifier–lecithin system, the emulsifier is heated with the lecithin and liquid oil before the mixture is dissolved in the melted fat blend. Additionally, lecithin reduces spattering in frying margarine, and it prevents burning of sediment compounds (lactose and casein), because lecithin incorporates these compounds. Lecithin is also a good antioxidant (Bonekamp 1990; Stauffer 1996).

Fat-soluble butter flavour and color (i.e., β-carotene) are added to achieve a product that tastes and looks like butter. In addition, β-carotene has pro-vitamin A activity.

Depending on geographical region, flavors can include a large variety of taste profiles such as fermented, buttery, fatty, vanilla, sweet cream, sour, and fruity.

Antioxidants can be added in order to improve the shelf-life of the product, because these substances can delay the onset or slow the rate of oxidation. In margarine production the following antioxidants can be added to the water or fat phase according to solubility: natural tocopherols, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), TBHQ (tertiary butyl-hydroquinone), ascorbic acid, and ascorbic palmitate (Danisco 2002c). The use of food additives, such as emulsifiers, flavors, colors, and antioxidants, is controlled by health authorities and governmental agencies. The use and the maximum dosages permitted in food vary. Thus, local legislation should always be consulted before using food additives in food products.

31.2.2 Water Phase

The water phase differs depending on the type of margarine; thus, it has a certain influence on the finished margarine. It consists mainly of water in which the minor ingredients such as salt or brine, milk or milk protein, and preservative are dissolved. For low-fat margarine, stabilizers can be added as well as water-soluble flavor and color. For puff pastry margarine, sugar can also be added.

Salt or brine is added primarily to improve taste, but also to prevent growth of micro-organisms. In frying margarine, salt helps to prevent spattering. A content of 1% salt will minimize the growth of the majority of micro-organisms in margarine (Danisco 2002b). The salt content varies from area to area of the world. For example, in Europe a salt content of 1–1.2% is normal, whereas in South America a salt content of up to 3% is not unusual.

Milk proteins such as skim milk powder, whey powder, cream powder, or sweet butter milk powder have an oil in water (o/w) emulsifying effect. These ingredients work against the margarine w/o emulsifier system, and thus destabilize the margarine emulsion. However, this enhances the flavor release, and milk protein is added to the emulsion when producing table margarine, reduced-fat spreads, and low-fat spreads to improve the taste. Additionally, milk proteins contribute to the desired browning effect in margarine used for frying (Gerstenberg and Agger 2001).

Citric acid is used to lower pH, which has the effect of not only extending shelf-life of puff pastry margarine, but it also contributes to lift of the puff pastry (Alexandersen 1996). Other preservatives often used in margarine are benzoates and sorbates. They are most active at a pH of $\sim$4.5; this makes the advantage of their addition doubtful, because the pH in most margarine emulsions is often close to neutral. In full-fat products, preservatives are not necessary as long as the product is properly crystallized and packed. If the water droplets are finely distributed and their size is between 2 and 4 μm, the possibility of occurrence of micro-organisms is limited (Danisco 2002b).

31.3 CRYSTALLIZATION TECHNOLOGY

Fats tend to crystallize in various forms having different melting points. Each of these crystalline forms with their respective melting points is called a polymorph and the phenomenon is called polymorphism (Timms 1984, 1985).

The triglycerides exhibit, with some exceptions, three basic crystalline forms designated alpha (α), beta prime (β'), and beta (β). In general, transformations take place in the order: $\alpha \rightarrow \beta' \rightarrow \beta$. The transformations are irreversible except by melting and

recrystallization. It is possible that the transformation from one polymorphic form to another takes place in the solid state without melting. This transformation will only take place in the direction of a more stable form seeking the most compact crystal form and the lowest thermodynamic energy state possible (Sato 1988).

Different polymorphs can co-exist in the fat. These different forms show melting points depending on the cooling and heating history of the fat. Due to a so-called crystal memory, crystalline structure is preserved even though the fat is melted. This structure will affect crystallization directly, especially when the rate of cooling is high (Larsson and Friberg 1990). The polymorphic changes in the margarine may lead to a grainy structure (Merker and Wiedermann 1958; Timms 1984; Johansson and Bergenståhl 1985).

When cooling the melt, α crystals are generally formed, but this form is never stable in triglycerides and transformation to β′ is a reality. In most cases, β′ crystals are relatively slowly transformed to the stable β form. The time of transformation from one crystal form to another depends on the composition of the triglycerides and the presence of diglycerides in the fat blend (Ong and others 1995). However, some fats possess both β′ and β forms, others only either the stable β′ form with no further transition or the stable β form.

The literature states that the degree to which fats will exhibit either β or β′ tendency depends on hydrogenation and depends to a lesser extent on blending factors (Table 31.2). Also, the palmitic acid content of the fat seems to be responsible for exhibiting a particular crystal habit. Fats containing a relatively low amount of palmitic acid (C16:0), approximately 10%, seem to be β tending, whereas fats with at least 20% palmitic acid, in general, are β′. It is not only the amount of palmitic acid that seems to determine the crystal habit, but also the distribution in the glycerol molecule. For instance, lard and tallow contain approximately 24 and 25% palmitic acid, However, lard is β tending due to the high concentration of palmitic acid in the sn-2 position of the glycerol molecule, whereas tallow is β′ tending because of sn-1,3 positioning (Wiedermann 1978).

At ambient temperature, natural fats contain both a liquid and a solid phase. A phase is a state of a homogeneous matter separated from other phases by a physical barrier, and can be defined by its composition, temperature, and pressure. Phases can be solid–liquid just like ice and water, oil and fat; phases can also be solid–solid when fat has co-existing polymorphs, and liquid–liquid as in mayonnaise and salad dressing.

It is important to have knowledge about phase behavior of fats because macroscopic properties such as spreadability of margarine and butter (Narine and Marangoni 1999) can be affected when different solid phases are present. The desirable properties of margarine are closely related to the kind of crystals present; β′ crystals are the most desirable apart from a few types of shortening (Wiedermann 1978). The β′ crystals are relatively small, exhibiting a needle-like structure, and can incorporate a larger amount of liquid oil in the crystal network. β′ crystals result in a glossy surface and a smooth texture.

TABLE 31.2 Crystal Habits of Selected Fats (Wiedermann 1978).

β′ Tending Fats	β Tending Fats
Butter oil/milk fat	Canola
Coconut	Cocoa butter
Cotton seed	Corn
Modified lard	Lard
Palm	Soybean
Tallow	Sunflower

The crystallization of melted fat is the process of phase transition of molecules from liquid to solid state; the driving force is the difference between the melting point of the fat and the actual solution temperature (Grall and Hartel 1992).

The solid phase formed will consist of finely dispersed and generally microscopically sized crystals. The remaining liquid phase will fill up the interstitial space around the crystals. The crystals are interconnected by bridges and form a crystal network, also called the solid/liquid matrix (Johansson and Bergenståhl 1985; Hoffmann 1989).

The transition from the α form to the β' form takes place in the crystallization equipment. All fats tend to crystallize in the β' form when crystallization takes place in an SSHE plant. Fats that are not stable in the β' form due to its triglyceride composition will eventually transform into the β form.

31.4 MARGARINE PRODUCTION

As described in the introduction, margarine and other crystallized fat products are today produced by either the SSHE process or the traditional chilling drum process.

The chilling drum is cooled inside by ammonia or freon evaporation. A thin layer of emulsion ($\sim$0.1 mm) is placed on the cooling surface of the chilling drum; thus, cooling of the emulsion takes place rapidly without agitation. After one rotation the crystallized emulsion is scraped off as a thin film or as thin flakes. The produced flakes will have to rest in trolleys or silos in order to complete the crystallization process. After hours of resting the margarine flakes are kneaded in the vacuum kneading unit in order to achieve a margarine block exempt from air.

Because the chilling drum plant requires more space, is more labor-consuming, and less hygienic than the SSHE, the continuous SSHE is used as the crystallization method for the majority of fat products produced around the world. Today the chilling drum process finds only limited use, mainly for the production of puff pastry margarine products often containing animal fats. In the SSHE, a different and slower crystallization process takes place while the emulsion is agitated when compared to the crystallization process in a chilling drum plant. However, the complete crystallization process from melted emulsion to finished margarine or spread is faster, more hygienic, and more efficient for the SSHE process when compared to the traditional chilling drum process. Apart from the actual crystallization line, a modern manufacturing facility for margarine and related products will typically include various tanks for oil storage as well as for emulsifier, water phase, and emulsion preparation; the size and number of tanks are calculated based on daily capacity of the plant. The facility also includes a pasteurization unit and a remelting facility (Fig. 31.4).

The various oils, either as an oil blend or single oils, are stored in oil storage tanks (area 1) typically outside the production facility. Each tank is furnished with automatic temperature control and an agitator in order to secure a stable storage temperature above the melting point of the fat and to avoid fractionation of the oils, respectively.

Typically, the plant is supplied with liquid emulsifier and lecithin from the emulsifier storage tanks (area 2). Emulsifier from blocks, pellets, or flakes can also be added to the emulsifier tanks manually. All emulsifiers are preblended with liquid oil, typically in the ratio of 1 : 5 in order to ease handling and to secure homogeneous distribution of the emulsifier later in the fat phase and the emulsion. The emulsifier tanks can either be hot-water heated or heated with steam. When the emulsifier blend is ready, it awaits transfer to the premix tank.

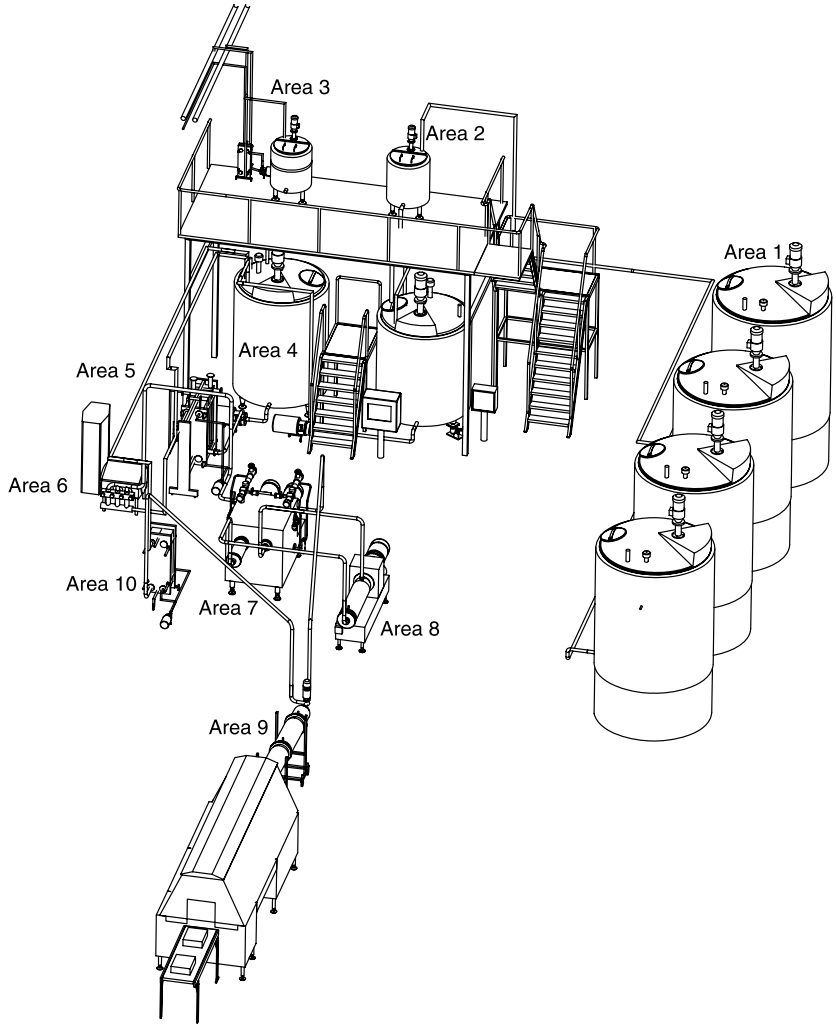

Figure 31.4 *Example of a margarine plant.*

For preparation of oil-soluble and/or water-soluble minor ingredients, smaller tanks are used. Minor ingredients such as salt, flavors, colors, preservatives, additives, antioxidants, and vitamins are dispersed later in the phases according, to solubility. The extent to which additives may be used in food as well as the maximum dosage permitted often vary from country to country. Therefore, the legal authorities should always be consulted before using additives in food products. The tanks for minor ingredients are connected to the water phase and premix tanks. The minor ingredients await the signal from the control system, which then automatically transfers the ingredients into the water phase or premix tanks.

Batching of the water phase in the water phase tank (area 3) can take place from four sources: water, milk, brine, water-soluble minor ingredients.

Water is supplied directly as sufficient pressure and capacity should be available. It must be of good drinking quality, free from harmful micro-organisms, and of an

acceptably low hardness as $CaCO_3$, that is, <100 mg/L (Bylund 1995). Normally, a water heater is included to ensure adequate temperature for dissolving the ingredients and to avoid precrystallization in the premix when the water phase is added to the oil blend.

Normally, milk is mixed using water and milk powder. This mix is dissolved, pasteurized at a temperature of at least 72°C for 15 s, and cooled to 5°C before transfer to the milk storage tank. As long as the milk has been produced from raw materials of sufficiently high quality (i.e., low number of colony forming units, CFU), and under good technical and hygienic conditions, the pasteurized milk should have a shelf-life of up to 4 days in the milk storage tank at 5°C (Bylund 1995; Larsen 2004).

Brine can be made in a locally supplied salt saturator of sufficient capacity. In order to achieve a steady flow in the salt saturator and avoid dragging unsaturated water through the saturator, a buffer tank is typically included. By using brine, the salt crystals are not present in the product, which positively affects the quality of the margarine or spread. In addition, the chilling tubes of the SSHE are very sensitive to salt, because salt is aggressive towards the coating materials used on the surface of the chilling tubes.

Minor ingredient units can be built for central preparation of water-soluble minor ingredients, and each of the units would be connected to the water tanks. The minor ingredient preblend could be dosed through a common pipe, then flushed with water and finally purged with N_2 to secure a clean pipe.

Oil and the emulsifier blend are typically metered by flow meters and/or weighed into the premix tank standing on load cells. The type of agitator used in the premix tank depends on the type of product; typically the impeller type or the anchor type are used. The agitators are specially designed to create a strong current that follows the shape of the tank. This efficient mixing assures that the product reaches everywhere in the tank and pushes the product upward, thus avoiding product dead zones. The agitator creates a uniform and controlled flow pattern for the entire batch.

Batching of emulsion takes place by transferring various oils and fats or blends to the premix tank (area 4) in which the emulsifier and other oil-soluble minor ingredients are added. When all the ingredients for the fat phase have been properly mixed, the water phase is added and the emulsion is created under intensive but controlled mixing.

When the emulsion is ready, it will automatically be transferred from the premix to the buffer tank to enable a new batch to start automatically in the premix tank. A solution with two combined premix/buffer tanks could also be an option where each tank would work as a premix tank as described above. Each tank would additionally work as a buffer tank, thus the line would be fed from one tank while a new batch would be prepared in the other.

From the buffer tank the emulsion is normally continuously pumped through the plate heat exchanger (PHE) or in some cases an SSHE for pasteurization prior to entering the crystallization line (area 5). The buffer tank receives the excess product returning from the remelt tank, remelt PHE (area 10), or remelt SSHE, which enables the remelted emulsion to be reprocessed. For very-low-fat products, the pasteurization process in the PHE might only involve the water phase, or if the complete emulsion is pasteurized it can be done in an SSHE due to the often high viscosity of the low-fat emulsion.

The emulsion is pumped to the crystallization line by means of a high-pressure piston pump (area 6). Apart from the buffer tank(s), PHE, and the high-pressure piston pump, a typical crystallization line for the production of margarine and related products consists of a high-pressure SSHE (area 7), pin rotor machine(s) (area 8), and a resting tube (area 9), as shown in Figure 31.5.

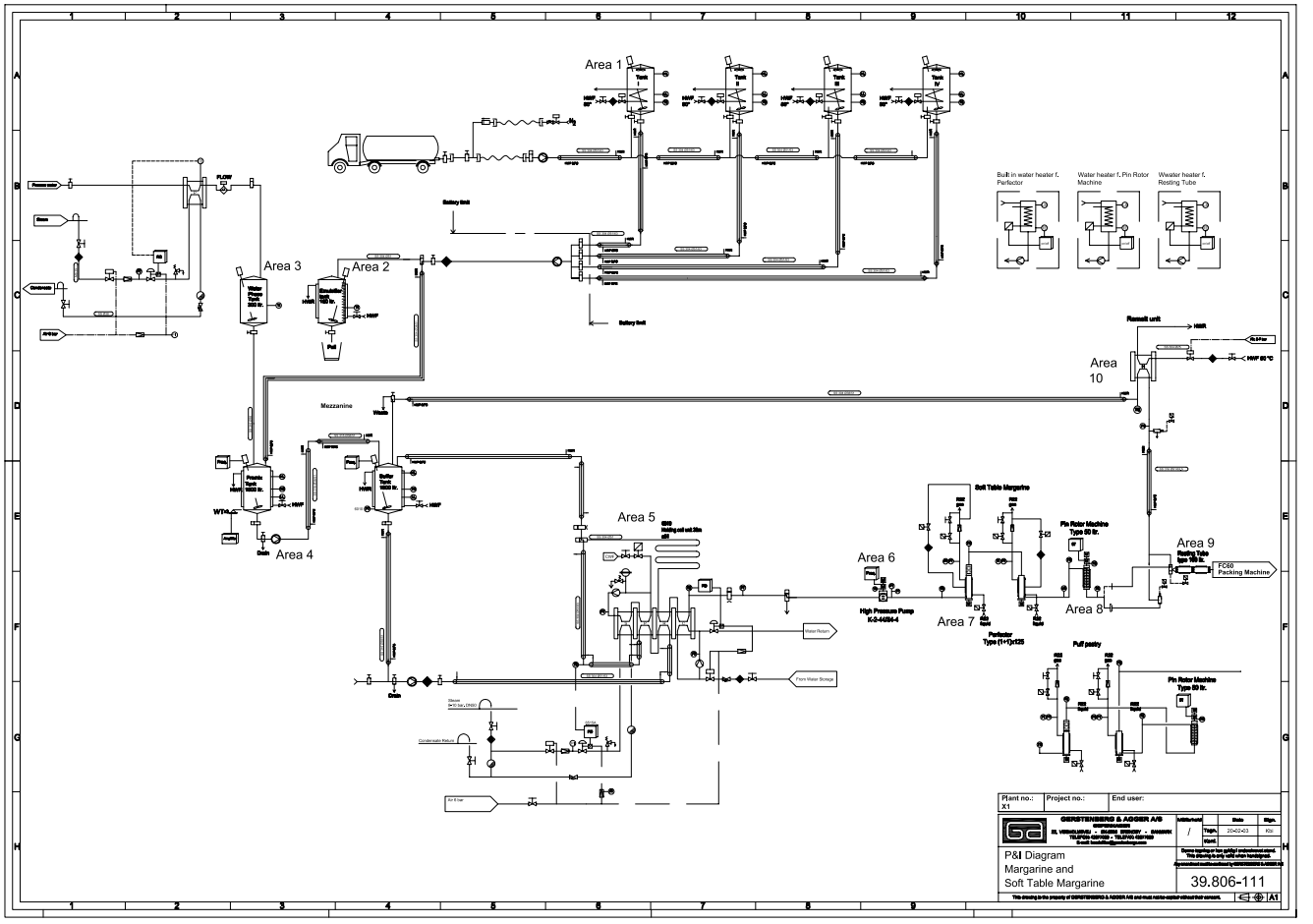

Figure 31.5 *P&I diagram of a margarine plant.*

Depending on the type of product to be manufactured, the configuration of the crystallization line (i.e., the order of the chilling tubes and the pin machines) can then be adjusted to provide the optimum configuration for the particular product. The heart of the crystallization line is the SSHE, in which the emulsion is chilled and then crystallized. As the crystallization line usually manufactures more than one specific fat product, the SSHE often consists of two or more cooling sections or chilling tubes in order to meet the requirements for a flexible crystallization line. When producing different crystallized fat products of various fat blends, flexibility is needed because the crystallization characteristics of the blends might differ from one blend to another.

After the product is chilled in the SSHE it enters the pin rotor machine where it is kneaded for a certain period of time and with a certain intensity in order to assist the promotion of plasticity and/or softness. If the product is meant to be distributed as a wrapped product it, will enter the SSHE again for a given period of time before it settles in the resting tube prior to wrapping. If the product is to be packed in cups, no resting tube is included in the crystallization line. Examples of various configurations of the crystallization line related to the type of product are shown in Figure 31.6 and will be described later in the section concerning industrial products and consumer margarine and spreads.

The crystallization process and the processing conditions have a great influence on the characteristics of the final margarine and spread products. When designing a crystallization line, it is important to identify the characteristics of the products planned to be manufactured on the line. To secure investment for the future, flexibility of the line is necessary, because the range of products of interest might change with time as well as the raw materials.

The values in Table 31.3 concerning residence time through the various units in the crystallization line are used when designing a crystallization line. These figures are also

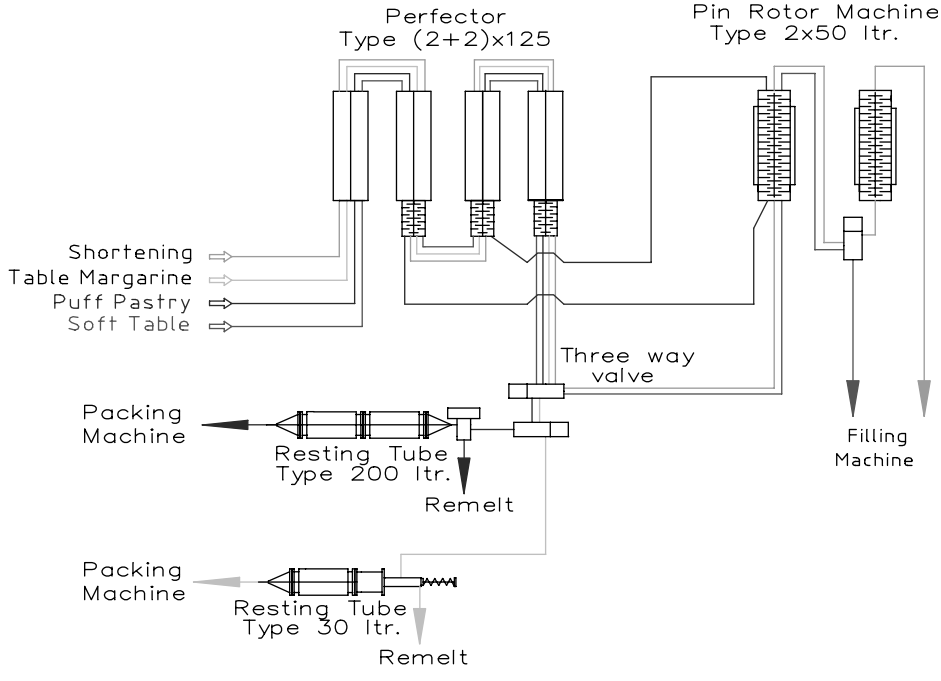

Figure 31.6 *Configuration of a flexible crystallization line (Gerstenberg and Agger 2001).*

TABLE 31.3 Recommended Processing Values for Residence Time Through Crystallization Unit Operations (Gerstenberg 2002, 2004).

Product	Pin Machine (% Volume/Time)	Resting Tube (% Volume/Time)	Capacity (%)
Puff pastry margarine	5–6	10–15	40–70
Cake and cream margarine	1–4	2	90–110
Table margarine	1–2	1–2	100
Soft table margarine	3–4	–	100–110
Low fat spreads	3–4	–	50–90

used in order to find the optimum processing parameters when manufacturing margarine and spreads products. When producing soft table margarine, lines are typically operated at full capacity. The cooling surface of the SSHE determines the capacity or product throughput of the margarine line. If the SSHE is designed to give 3000 kg/h soft table margarine, as a rule of thumb the volume of the pin rotor machine will be 3–4% of the throughput, meaning 90–120 L. The specific volume of pin rotor machine used depends on the type of soft table margarine and is decided individually from plant to plant.

The HPP feeding pump is not shown, but the SSHE, intermediate crystallizers (I/C), pin rotor machines (PRM), and resting tubes (RT) are shown in Figure 31.6. These are the necessary units to produce a large number of margarine and spread products. In this case a $(2+2)\times 125$ Perfector (manufactured by Gerstenberg and Agger A/S, Denmark) is used as SSHE and this type has a nominal capacity of 750 kg/h/tube measured by 80% margarine. Thus, the line can manufacture 3000 kg/h. It is named "2 + 2" because each cooling section has two chilling tubes, and "125" because the diameter of the chilling tube is 125 mm. Each chilling tube has a cooling surface of 0.42 m^2, resulting in 1.68 m^2 for the complete line (Gerstenberg and Agger 2004). The actual capacity of the line is determined by the cooling surface available and the type of product being manufactured.

In the Perfector, the emulsion is cooled and the crystallization process is initiated when the warm liquid emulsion meets the cold cooling surface on the inner wall of the SSHE. The emulsion is subsequently kneaded in small kneading units, I/C, mounted directly on the Perfector shaft or in the larger volume PRM. In these kneading units the secondary nucleation is promoted along with the plastic secondary bonds. In the PRM a rise in temperature will occur due to the release of heat of crystallization and mechanical heat. Typically, if the product is filled in cups or bag-in-box, the PRM will be included in the line prior to the filling machine as shown with soft table margarine, for example. If the product is packed or wrapped, the last step in the crystallization line will be the RT, as shown for table margarine.

Thus, the configuration suggestions shown in Figure 31.6 used in connection with the recommended figures in Table 31.3 provide a good foundation for optimal processing conditions. In the following section, characterization of various crystallized fat products will be described.

31.5 INDUSTRIAL PRODUCTS

Industrial products include shortenings and bakery margarine. Shortenings are defined as 100% fat products and will not be described in this chapter. Bakery margarine includes

products such as puff pastry margarine, cake margarine, and cream margarine. These products typically exhibit a fat content of 60–80% with the high-fat products still being most common.

Puff pastry margarine or puff pastry butter is used for the production of puff pastry and various baked products, which are characterized by a flaky structure of good volume and uniform appearance (Kun and Ong 1986). The flakiness depends partly on the plasticity of the roll-in puff pastry margarine, because the margarine in connection with a basic dough is rolled and folded several times in order to produce a thin product containing multiple, alternate dough/margarine layers. In order to achieve baked products with a flaky structure, formation of thin films of gluten that trap water vapor and carbon dioxide from fermentation is necessary. The thin films of gluten can form a three-dimensional structure. To prevent this from occurring, the laminates must be separated by continuous sheets of fat. Thus, the fat works as a barrier between the basic dough layers and prevents these from joining during the layering procedure. To function as a barrier the fat should not be absorbed by the dough layers and it must remain as a continuous film throughout the procedure.

The main demands of puff pastry margarine and butter are plasticity and firmness, because soft and oily margarine tends to be absorbed by the dough, and hard and brittle margarine is difficult to stretch during the abovementioned layering procedure. In both cases the baking performance will be affected negatively (Larsen 2004).

From Tables 31.3 and Figure 31.6 it can be noted that puff pastry margarine or butter is produced at a lower capacity (throughput rate). The content of solids is relatively high at usage temperature (high SFC profile), as it is important that the margarine is in its solid form during the layering procedure and before the baking process. The relatively high amounts of solids at given temperatures result in a need for more cooling energy in order to achieve crystallized products at packing. In addition, longer kneading time in the pin rotor machine(s) and SSHE is needed to achieve the plasticity and firmness of the product; therefore, a longer residence time is needed in the crystallization line. Typically, puff pastry margarine is produced with the configuration shown in Figure 31.6. The PRM is positioned between the two cooling sections in order to give the fat time to crystallize without cooling, but while kneading, which results in maximum plasticity in the product.

Traditionally, hydrogenated fats were used to produce puff pastry margarine, but palm oil and its fractions are now used to a great extent to minimize trans fatty acid content. It is additionally reported in the literature that puff pastry margarine based on palm oil shows excellent plasticity (deMan and deMan 1994). In some areas of the world where animal fats are still natural to use and available at favorable prices, beef tallow can be used for the production of puff pastry margarine. Also, interesterified blends can be used for the production of puff pastry margarine with success. However, the abovementioned recommended processing figures with regard to residence time and configuration have to be reconsidered, because margarine made by these blends tends to become greasy easily (Kirkeby 2003).

Cake and cream margarine can be covered as one, because often the same margarine is used for both of these applications. For example, this type of margarine is used in pound cake, short cake, and filling or decoration cream.

Cake margarine should exhibit a short structure in order to ensure that the final baked products have good crumb structure. The function of cake margarine is to prevent the formation of the gluten network as described under puff pastry margarine.

At usage temperature, cake margarine should consequently be soft and easy to incorporate into the batter in order to apply optimal stability to the finished products. Hereby, the fat will coat the individual flour particles and prevent these from forming the gluten network.

Firmer and more plastic cake margarine is used in cookie production where no air is incorporated into the batter (Danisco 2002a). Easy incorporation of the fat into the batter is still important and a certain plasticity of the margarine is necessary in order for the cookie to keep its shape and not spread out before baking.

At usage temperature, cream margarine should exhibit a short structure like cake margarine and the consistency should allow a large volume of air to be sufficiently finely incorporated and maintained. The cream must not collapse or separate on standing. Tempering is utilized to obtain the correct consistency.

From Table 31.3 it can be noted that cake and cream margarine can be produced with relatively low or high amounts of PRM volume. As a rule of thumb, packed cake and cream margarines require a lower amount of PRM volume positioned between the cooling sections and will settle in the RT before packing. Cake and cream margarine that is filled in boxes needs the PRM between the cooling sections for the plasticity but also at the end prior to the filling machine for plasticity and uniform filling.

It is generally accepted that lauric oils such as coconut and palm kernel oil (containing lauric acid) provide good whipping properties. These oils crystallize quickly due to a relatively steep melting profile or SFC profile, and they form small crystals in margarine, which ensures a homogeneous product. Additionally, the fat blend contains a certain proportion of higher melting fat and liquid oil in order to achieve strength in the crystal network and easy whipping ability, respectively. The whipping ability has been shown to be inhibited by fat blends containing a large number of triglycerides with similar fatty acid chain length such as hydrogenated rapeseed and hydrogenated sunflower oil. These fats also transform easily into the β crystal form. Interesterified fats can be used for cake and cream margarine (Danisco 2002a).

Industrial margarine is evaluated according to application. Margarine used for baking is typically subjectively evaluated for plasticity, and objective baking tests are used that involve a standard baking method imitating the application. Margarine used for creaming purposes is evaluated for creaming performance. Margarine is whipped with sugar according to a standardized procedure and the ability to incorporate air is calculated by weighing the known volume. Low specific volume is ideal.

Flavour is important for all types of margarines. In the case of margarine used for baking, the flavor has to exhibit certain heat stability or carry-through in order not to disappear completely during baking. Special carry-through flavor systems exhibiting a butter-like taste with necessary heat stability are on the market. Flavors for cream margarine usually have a light delicate butter experience (Danisco 2002a).

31.6 CONSUMER MARGARINE, SPREADS, AND DAIRY SPREADS

Consumer margarine, spreads, and dairy spreads cover a range of semifirm to soft products such as table margarine, soft table margarine, and low-fat spreads. Margarine is by definition a product containing 80% fat, whereas spreads are defined as products with a fat content lower than 80%. Reduced-fat spread contains approximately 60% fat, low-fat spread contains 40%, and very-low-fat spread has the range 20–35% fat content. Dairy spreads are defined as products containing a significant amount of butter or butter fat.

Table margarine is used in households or in food service as a substitute for butter in applications that include spreading on bread, baking, and pan frying, for example. Table margarine is typically marketed in a block format, often wrapped in foil or paper; the block format necessitates that this product be relatively firm in consistency in order to shape. However, a certain plasticity is necessary due to the multipurpose nature of the product. Depending on the climate or season, firmer and softer variations occur around the world. In Figure 31.3 it can be seen that table margarine does not show a straight SFC profile due to the fact that the product needs to melt relatively fast in the mouth. In addition, the product should exhibit a certain firmness and plasticity; thus, table margarine is formulated to have relatively high amounts of solids at 15°C.

According to Figure 31.6 and Table 31.3 table margarine is produced at nominal capacity without the PRM. However, the PRM is replaced by I/Cs in this case. The I/Cs shown in Figure 31.6 each have a volume of 15 L; thus, the total kneading volume in the crystallization line is 45 L, corresponding to 1.5% of the line. The I/Cs could be replaced by one, 50-L PRM in order to have the possibility of altering the rotation speed of the kneading unit. A relatively small volume of RT is needed due to the fact that the blends normally used for table margarine crystallize fast and only need a short time to firm prior to extrusion or packing.

Traditionally, the higher melting portion of the margarine fat blend consists mainly of partially hydrogenated vegetable oil. In general, hydrogenated fats crystallize relatively faster than unhydrogenated fats and provide the finished margarine with the required characteristics (Yap and others 1989). However, during the hydrogenation process, various trans fatty acids are formed and at present these isomers are thought to be nutritionally undesirable. Several studies seem to indicate a correlation between a certain intake of these isomers and the risk of cardiovascular diseases. The main direct sources of trans fatty acids in the human diet are margarine and crystallized fat products. Indirect sources are contributed by products such as baked products, fried foods, and so on (Mattson and Grundy 1985; Boyeens and others 1988; National Research Council 1989; Mensink and Katan 1990; Emken 1991; London 1991; Nestel and others 1992; Mensink and others 1992a,b; Willet and others 1993; Wood and others 1993; Ascherio 1994; Danish Nutrition Council 1994; Judd and others 1994).

However, it is possible to replace the trans-fatty-acid-containing, partially hydrogenated fats by fractionated or interesterified intermediates. These fats will still fall into the two main groups of semisolid and hard stock fats as shown in Table 31.1. Due to the abovementioned trans fatty acid issue, the majority of the larger margarine producers around the world have shifted from high to low or non-trans-fatty-acid-containing blends; legislation in Denmark, for example, states a 1% limit of trans fatty acids (Watkins 2004). Today, palm oil, palm oil fractions, and interesterified blends are used to a great extent in the production of margarine.

Soft table margarine is used mainly for spreading on bread. It is a softer version of table margarine, and consequently contains a larger amount of liquid oil and exhibits a lower SFC profile. Soft table margarine is spreadable directly from the refrigerator but should still have a certain texture. Soft table margarine is relatively easy to produce; however, a large variety of soft products containing functional ingredients are on the market and these products can have special processing demands. An example would be production of a spreadable product containing stanolesters (Holmström 1997), which are claimed to lower the cholesterol levels in the blood of humans by 14% at a daily intake of 2 g. Initially manufactured and sold on the Finnish market, similar products are now available

in many countries around the world where heath claims associated with margarine products are not restricted by legislation.

Soft table margarine is usually produced by applying PRM at the end of the processing line as shown in Figure 31.6. This process step ensures that excess heat of crystallization is removed, which prevents the margarine from becoming brittle. The PRM at the end also ensures a uniform filling in the cup. However, if the soft table margarine is made by a high-liquid–low-solid blend, it can be advantageous to place the PRM between the two cooling sections avoid mechanically overworking the margarine prior to filling.

Low-fat margarine is mainly used for spreading on bread, but reduced-fat spread can additionally be used as traditional table margarine. Production of reduced fat spread is very similar to the already mentioned table and soft table margarine. The trend towards reduced- and low-fat products can be explained by an increasing interest from consumers due to an increasing awareness of their fat intake (Merker and Wiedermann 1958).

Low-fat margarine exhibits a higher water content than 80% margarine, which sets special demands on the emulsifier system and processing. The emulsifier should be able to bind large amounts of water in order to obtain a stable product with a narrow water droplet distribution; this results in a spread with a long microbiological shelf-life. However, a too narrow water droplet distribution is not necessarily an advantage, because this tight emulsion will affect the taste impression. Therefore, milk protein is often added to low-fat spreads, because they destabilize the emulsion and act as an o/w component that results in a more open emulsion. In order to control the emulsion when milk protein is present, hydrocolloids are added (Chrysam 1996; Gerstenberg and Agger 2001; Danisco 2002d).

There is a wide range of hydrocolloids, stabilizers, or textural ingredients on the market for use in margarine and dairy spreads. Water-binding capacity and sensory properties have to be considered when the manufacturer chooses a stabilizer.

Gelatine has a bland taste and melts at body temperature like a typical table margarine oil blend. For these reasons it has been preferred by many manufacturers for years. However, in recent years margarine manufacturers have been reluctant to use gelatine due to its association with bovine spongiform encephalopathy (BSE) and possible other considerations like kosher or lifestyle issues. Alginates, carrageenans, pectins, and starches all have a good water-binding effect and give stable emulsions, but all leave different taste impressions. Additionally, various mixtures of the abovementioned stabilizers are on the market, offering margarine producers a great variety of stabilizer systems.

Low-fat spreads are produced at different capacities as shown in Table 31.3. Although capacities are desired, some emulsions are so sensitive to shear forces that capacity must be decreased to avoid emulsion separation. Low-fat spreads are usually made with high-liquid–low-solid blends and the PRM is often positioned between the two cooling sections for the same reasons as described in the section concerning low-fat spreads. However, for some low-fat emulsions the optimal configuration of the crystallization line is obtained by having the PRM at the end of the line prior to the filling machine. The filling temperature of low-fat spreads is relatively higher than for soft table margarine, for example, due to the higher viscosity of the low-fat emulsion. If the filling temperature is too low, the final product may become brittle and crumbly, with free water as a result (Gerstenberg and Agger 2001; Danisco 2002d).

Dairy spreads cover products containing butter and vegetable oils and/or vegetable fats at various ratios with a total fat content less than 80%. Typically, dairy blends will cover the product group of 80% fat products, and the dairy spreads will cover the product groups

of reduced fat (60% fat) and low fat (40% fat) (Merker and Wiedermann 1958). Dairy blends have been on the market in northern Europe since 1969 (Merker and Wiedermann 1958; Mortensen 2001), but today the products are sold all over the world. Dairy blends were initially developed in order to meet consumers' demands for butter-flavored products spreadable directly from the refrigerator.

Dairy blends are produced batchwise in a butter churn or via a continuous process in a butter-making machine. This method has the disadvantage that it is difficult if not impossible to produce dairy spreads with a lower fat content than 60%. The dairy blends produced by this method exhibit a consistency very similar to butter, but tend to be softer as liquid oil has been added (i.e., lower SFC profile). However, in this chapter, only dairy blends and dairy spreads produced by margarine technology will be described.

Dairy blends are used domestically for spreading on bread, baking, and pan frying, whereas dairy spreads are mainly used for spreading on bread due to the higher water content. Dairy spreads can be a challenge to produce using SSHE technology with respect to obtaining plastic texture. However, the challenges can be minimized by optimizing the fat blend. For example, this can be done by adding anhydrous milk fat (AMF) to the blend instead of only depending on butter and/or cream as the fat phase. Apart from the butter fat and liquid vegetable oil, some dairy blends and spreads contain partially hydrogenated oil or interesterified fat.

When producing dairy blends and spreads in the SSHE plant, margarine technology is used. The fats for the fat blend are melted and the liquid oil, including possible emulsifiers, is added along with the water phase. The products are produced according to the configuration shown in Figure 31.6 and Table 31.3 for similar margarine products. However, the maximum recommendation values in regard to kneading volume are normally implemented in order to obtain products with a satisfactory texture and plasticity. Often it is necessary to reduce capacity (throughput) as well.

Apart from the margarine method, other processing methods are also used in the industry today. These processes can include a combination of the traditional butter manufacturing process and the SSHE process (Merker and Wiedermann 1958; Jensen and Kirkeby 2003).

Consumer margarine and dairy spreads are all evaluated according to the same parameters, because these products are used for the same purposes. Appearance, texture, spreadability, taste, and shelf-life are normally the most important parameters in regard to consumer margarine and spreads. However, an antispattering test is normally conducted for consumer products such as table margarine, reduced-fat spread, and dairy spreads that are used for frying. For low-fat spreads the free water stability is additionally evaluated.

The appearance of margarine and spreads is described by evaluating the following characteristics of the surface of the product: shiny, greasy, dull, dry, even, uneven. It is important that the product appears attractive. Thus, filling or packing has to be performed satisfactorily.

Texture is evaluated by describing the hardness and plasticity of the product. There are objective analyses available for measuring hardness and plasticity such as the Texture Analyser (Stable Micro Systems, UK) (Stable Micro Systems 2004). However, more simple and subjective methods are still in use around the world.

Spreadability is a very important quality parameter for consumer margarine, spreads, and dairy spreads. The homogeneity, consistency, gloss when spreading, sandiness, and free water can also be determined. The stability of the products can be described by a spreadability test that involves the spreading of a sample with a knife on cardboard in

order to imitate the spreading on a piece of bread. The abovementioned parameters can therefore be easily observed. The risk of free water is usually limited to low-fat spreads.

Taste is relatively important for all products, and as a general rule the taste impression of low-fat spreads should be similar to their related full-fat products in terms of melt down and flavor release. As described earlier, taste preferences differ around the world; thus, melt down and flavor release are individually described as fast, average, or slow.

Shelf-life can be determined in terms of microbiological or oxidative shelf-life. Also, for some products a stability test can determine the shelf-life. The microbiological shelf-life is increased with smaller water droplets. Oxidative shelf-life is increased by using high-quality oils and fats, and is further improved by adding antioxidants to the fat phase (Danisco 2002d).

31.7 PROCESSING AND PRODUCT TRENDS

New products are introduced into the market every year, and these products may require processing developments in order to be successfully produced. Demand for low- and non-trans-FA products seems to be of worldwide interest. In North America the concern is more focused on low- and non-trans-FA products due to the traditional use of partially hydrogenated soybean in margarine. Europe has a demand for genetically modified organism (GMO) free products and functional foods, and there is also demand for low-fat butter spreads. In South America interest is high for very-low-fat spreads (20% fat content). For environmental and economical reasons, production of cartonless butter products is of interest for the butter-producing countries and methods are available on the market today. After the traditional butter-manufacturing process, the butter can be chilled further in an SSHE, formed, wrapped in foil or plastic, and subsequently stacked on the pallet without the cardboard box (Kirkeby 2001). The abovementioned hot topics should only be taken as examples from a long list of trends around the world.

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32

Cream Products

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32.1 Introduction 726
32.2 Composition of Cream 726
32.2.1 Lipids 726
32.2.2 Proteins and Enzymes 726
32.2.3 Milk Fat Globule Membrane 727
32.3 Structure of Cream 727
32.3.1 Emulsion 727
32.3.2 Globule Size Distribution 727
32.4 Cream Preparation 727
32.4.1 Separation 728
32.4.2 Heat Treatment 728
32.4.2.1 Pasteurization 728
32.4.2.2 Heat Stability 729
32.4.3 Homogenization 730
32.4.3.1 Types of Homogenizers 730
32.4.3.2 Effect on Physical Properties 731
32.4.3.3 Lipolysis 732
32.5 Stability 733
32.5.1 Rupture 734
32.5.2 Clustering 734
32.5.3 Clumping 734
32.5.4 Coalescence 734
32.5.5 Creaming 735
32.6 Physical Properties 735
32.6.1 Rheological Properties 735
32.6.2 Effect of Cooling 738

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

32.6.3 Re-Bodying Effect 738
32.6.4 Agglutination 739
32.7 Types of Cream 739
32.7.1 Whipping Cream 740
32.7.2 Sterilized Cream 742
32.7.3 UHT Processed Cream 743
32.7.4 Recombined Cream 744
32.7.5 Cream Liqueur 744
32.8 Volatile Components 744
32.8.1 Off-Flavors 745
32.9 Summary 745
References 747

32.1 INTRODUCTION

Cream is an oil-in-water (o/w) emulsion of milk fat containing fat globules coated with native milk fat globule membrane (MFGM) material. This protective layer reduces both the extent of lipolysis and oxidation of the fat (Chen and Nawar 1991), and the extent to which globules aggregate and rise to the surface to form a cream layer. Surrounding the fat globules in an aqueous serum phase are soluble components such as salts, organic acids, whey proteins, other nitrogenous compounds, and lactose, as well as dispersed casein micelles in suspension.

This chapter will describe physical and chemical changes that take place during processing of cream and how these impact upon functionality. For more detailed information on the chemistry and physics of the milk fat globule, the reader is directed to other literature (Mulder and Walstra 1974; Wong and others 1988), and particularly Walstra and others (1999), from which some of the discussion on the physico-chemical properties of cream in this chapter are derived.

32.2 COMPOSITION OF CREAM

32.2.1 Lipids

The fat component of cream consists of a lipid core containing approximately 98% triacylglycerides, as well as the lipid components of the MFGM. Other relatively minor components of the lipid phase include diacylglycerides, monoacylglycerides, free fatty acids, sterols, and phospholipids. The most common fatty acids at levels greater than 10% in milk fat are oleic acid (C18 : 1), stearic acid (C18 : 0), palmitic acid (C16 : 0), myristic acid (C14 : 0), and butyric acid (C4 : 0). Milk fat melts completely at around $+40^{\circ}$C and is completely solid at -40°C, with about 50% solid fat content at 0°C.

32.2.2 Proteins and Enzymes

The serum phase of cream contains casein micelles and whey proteins, which retain their original composition in the aqueous phase after cream separation. The presence of casein in the serum phase can be exploited by the addition of a small amount of rennet to increase the firmness of cream.

Plasmin is an alkaline milk protease found associated with the casein micelle and is important in cheese ripening. Pasteurization increases plasmin activity by activating the transformation of the plasminogen precursor into plasmin. Cream also contains an acid milk protease associated with the casein micelle. More detailed descriptions on enzymes in milk can be found in Fox and McSweeney (2003).

32.2.3 Milk Fat Globule Membrane

The MFGM layer is a complex multilayered arrangement of protein, lipids, and other components at the fat–water interface (Danthine and others 2000) with a thickness of between 10 and 50 nm (Freudenstein and others 1979). The amount of membrane material adsorbed to the surface is inversely proportional to the square of the globule radius. The major protein components of the MFGM layer are butyrophilin and xanthine oxidase along with at least 30 identified enzymes. Approximately 25–60% of the MFGM is protein-based, with the remaining being mostly neutral lipids or phospholipids. The large variation is a consequence of different methods of assay and the washing procedures used to isolate MFGM material. A review of the nomenclature of the main proteins found within the MFGM layer is provided by Mather (2000). Other lipid-based components of the MFGM layer include cerebrosides, free fatty acids, sterols, sterol esters, and glycerides. Copper and iron are also found in trace amounts.

32.3 STRUCTURE OF CREAM

32.3.1 Emulsion

Cream contains fat globules dispersed within an aqueous continuous phase, and, as such, can be considered a fat-in-water emulsion. The globules have an overall negative charge, as shown by the negative zeta potential, ranging from −12 to −16 mV as the calcium concentration varies (Dalgleish 1984).

32.3.2 Globule Size Distribution

The fat globules in cream are mostly within the size range 0.1–20 μm. Larger globules will rise faster than smaller ones during the process of creaming. One gram of fat contains around 10^{11}–10^{12} globules, one-quarter of which are greater than 1 μm in size (Walstra and others 1999). Although larger globules are fewer in number, these constitute most of the fat in cream on a volume or weight basis. The average surface area weighted diameter (d_{32}) of bovine fat globules is around 3 μm, although this will vary across breeds, and certainly will vary according to the species of mammal. Ayrshire and Holstein milk contains larger fat globules, on average, than Jersey or Guernsey milk (Jenness 1988).

32.4 CREAM PREPARATION

Full-fat bovine milk contains an average of 4% fat, ranging from around 3.8% for Holstein milk up to around 5% for Jersey. Increasing the fat content of milk to around 12–80% is the essence of cream manufacture. This is commonly achieved by centrifugal separation; however, membrane processing can be employed to a similar effect. Centrifugal cream separators consist of concentric 60° cones stacked one on top of another. The cream

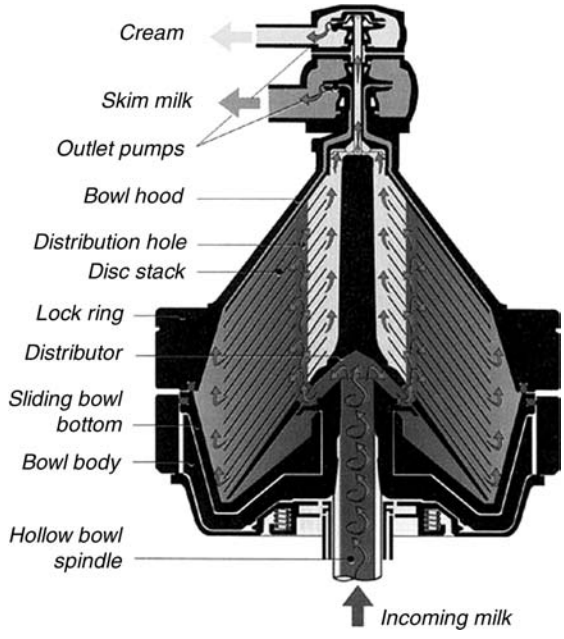

Figure 32.1 *Diagram of Tetra Centri hermetic separator (used with the kind permission of Tetra Pak).*

phase moves to the top of each cone under centrifugal force and exits the separator through a different outlet to the skim milk phase (see Fig. 32.1).

32.4.1 Separation

The basic steps involved in cream production are shown in the flow diagram in Fig. 32.2. With efficient separation, the fat content of skim can be as low as 0.05%. Factors that affect separation include temperature, centrifugation speed, cone configuration, and flow rate of milk. A review of the operation of a separator is given by Towler (1994). Stabilizing salts such as sodium citrate may be added at levels of around 0.15% prior to heat treatment to reduce the effect of heat instability during processing (see Section 32.4.2.2).

The temperature of milk separation affects the physical properties of the resultant cream. Cold separation (12°C) produces larger fat globules, higher viscosity, shorter whipping time, and higher free fat (perhaps due to more extensive shear damage) compared to separation at 50°C (Hillbrick and others 2000). There is also a greater concentration of phospholipids in the cream when separated at colder temperatures.

32.4.2 Heat Treatment

32.4.2.1 Pasteurization. Cream is usually heat-treated at 77–82°C for about 30–60 s. This is a higher temperature compared to that used in pasteurization of raw milk (72°C for 15 s). Heat treatments above 80°C for 20 s result in milk with a significant cooked flavor. High heat treatments will also denature α-lactalbumin and β-lactoglobulin, which will complex with the surfaces of casein micelles and fat globules (Corredig and Dalgleish 1996a,b). The time and temperature heating regime will also affect the functionality of buttermilk derived from the MFGM layer (Corredig and Dalgleish 1998).

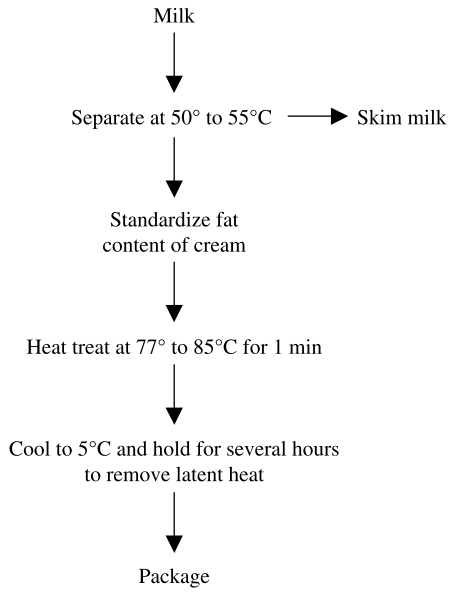

Figure 32.2 *Basic principles of cream separation (adapted from Walstra and others 1999).*

32.4.2.2 Heat Stability. Homogenization creates additional surface area that then must be coated with surface-active material to maintain stability against phase separation. Adsorption of casein micelles and micellar fragments effectively yields a fat globule that functions as a casein micelle with a lipid core. These casein-coated globules are capable of interacting with casein micelles in the surrounding aqueous phase. Higher protein content in the serum phase lowers heat stability by increasing the surface load of proteins on the fat globule after homogenization.

Heat stability decreases as both the amount of fat and the homogenization pressure increase. A larger number of fat globules and a higher homogenization pressure both result in statistically more collisions. Both of these effects will enhance heat-induced clustering.

The pH optimum of heat stability is near 6.7 (Walstra and others 1999). Processing conditions such as acidification, renneting, or high heat treatment of cream will result in decreased heat stability brought about by casein–casein surface interactions. This problem can be circumvented by addition of stabilizing salts such as sodium citrate or sodium hydrogen phosphate, which raise the pH and chelate calcium ions from the micelle, therefore reducing the propensity for interaction with homogenized fat globule surfaces.

Heat stability rises as temperature increases up to about 75°C, beyond which stability decreases (Mulder and Walstra 1974). As temperature increases to 75°C, the amount of adsorbed casein per unit surface area decreases due to spreading and breaking apart of micelles into smaller fragments. The lower surface concentration of caseins at the higher temperatures will promote a more stable fat globule. Above 75°C, whey proteins denature and adsorb to the fat globule surface, promoting fat globule interactions and thus reducing heat stability.

It is usually desirable for heat treatment to precede homogenization. The coating of fat globules with casein as a consequence of homogenization will decrease heat stability. The application of heat, particularly above 135°C, will promote casein-induced aggregation of

globule surfaces (van Boekel and Folkerts 1991). These aggregated fat globules will have a higher density than native globules due to the extra adsorbed casein and will sediment over time in UHT treated cream. To circumvent this problem, homogenization should preferably take place after UHT heat treatment.

Addition of sweet buttermilk, buttermilk powder, or monoacylglycerides enhances heat stability prior to homogenization. Both phospholipids and monoacylglycerides migrate to the interface and reduce the homogenization-induced adsorption of caseins responsible for heat instability. Lecithin improves the heat stability of recombined and conventional full-cream evaporated milk by interaction with the MFGM layer (McCrae 1999).

32.4.3 Homogenization

Homogenization of milk or cream is employed to break apart larger fat globules into smaller globules, largely to prevent creaming, or to incorporate newly adsorbed material to the fat–water interface. This is concomitant with an increase in the total fat–water interfacial surface area, which must be coated with additional surface-active material, primarily casein micelles and micellar fragments, to prevent large-scale coalescence and phase separation. Homogenization at a typical pressure of 20 MPa results in a thousand-fold increase in the number of globules, a ten-fold increase in the total surface area, and a ten-fold reduction in the average diameter.

Emulsions are thermodynamically unstable, but kinetically stable, meaning that globules remain as discrete entities for long periods of time. Aggregation is hindered by the presence of the surface-active membrane layer, whether it be native MFGM or an introduced emulsifier. The size of globules is dictated by many factors, not limited to the following:

1. Homogenization pressure;
2. Time between collisions of globules during homogenization;
3. Concentration of emulsifier;
4. Solubility of emulsifier;
5. The hydrophilic–lipophilic balance (HLB) number, or the amphipathicity of the emulsifier;
6. Conformation of the emulsifier;
7. Propensity for adsorption of the emulsifier at the surface;
8. Time for migration of the emulsifier to the interface;
9. Flexibility of the emulsifier on the surface after adsorption;
10. Solution conditions such as pH and ionic strength;
11. Temperature; and
12. Fat or oil content.

32.4.3.1 Types of Homogenizers. The process of homogenization introduces a kinetic barrier to phase separation in a bi- or multiphasic system. In dairy products, this usually means that fat is emulsified by coating with a surface-active component (such as proteins and phospholipids) and the resultant globules are dispersed in the aqueous serum phase. The surface coating serves to reduce surface tension, and also to provide a barrier against re-coalescence during homogenization where globules undergo rapid collisions.

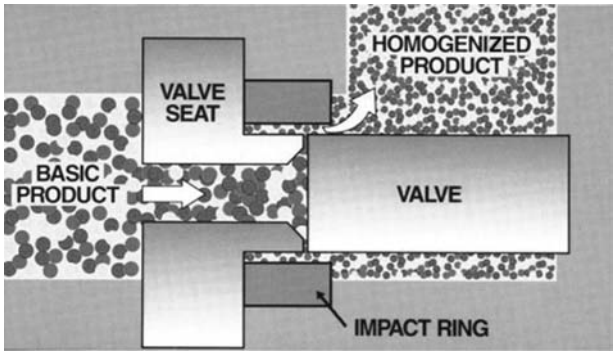

Figure 32.3 *Homogenizer valve (used with kind permission from Invensys APV).*

The energy to break apart large fat globules is introduced through turbulent flow and cavitation while the fluid product passes through a narrow orifice (homogenization valve) or into a high-speed blending device. Pressure can be adjusted by altering the distance of separation between the valve and the valve seat (see Fig. 32.3). An increase in the temperature of milk occurs during homogenization, approximately 1°C for every 4 MPa of pressure. Cavitation takes place where high-velocity fluid enters the homogenizer at a lower pressure, in accordance with the Bernoulli effect. This effect causes localized vaporization of the aqueous phase to form vapor bubbles that collapse in the homogenization valve under pressure, sending shock waves through the fluid and breaking apart fat globules.

Different types of homogenizers can be used to process fluid products such as cream or milk. High-speed blending does not create sufficient turbulence for this to be an effective process. High-pressure valve homogenizers are common in the dairy industry. These usually employ two stages of homogenization (Fig. 32.4) to create smaller globules at the higher pressure first stage, and to break apart fat globule clusters at the lower pressure second stage. Ultrasonic homogenizers utilize vibrating piezo-electric crystals or liquid jet generators. Fat globules can also be broken apart by membrane processing. Homogenization using a Microfluidizer™ involves a process of accelerating cream to a high velocity followed by separation into two streams with subsequent violent collision. Microfluidization can result in very high effective pressures in excess of 100 MPa, with fat globules sizes as low as 100 nm with a relatively narrow particle size distribution. Additional information on homogenizers and the homogenization process is provided by McClements (1999).

32.4.3.2 Effect on Physical Properties. Homogenization reduces the extent of creaming by several mechanisms. The first stage of a two-stage homogenization process increases the rate of fat globule collisions and promotes the formation of heat-stable clusters of globules. A typical first-stage homogenization pressure is around 15–20 MPa. A second homogenization stage at a lower pressure of less than around 5 MPa is sufficient to break apart clusters, but not to form new clusters. Homogenization also narrows the fat globule size distribution width, which minimizes creaming. Homogenization of milk with subsequent separation to a higher fat content will yield a narrow particle size distribution of globules with less creaming. Other effects of homogenization are to make cream whiter, enhance lipolysis in unpasteurized cream, slow down the rate of rennet reaction during cheese manufacture, increase the viscosity of yogurt, and inactivate agglutinin factors to prevent creaming. Viscosity of cream increases at a higher fat content, and also after first-stage homogenization

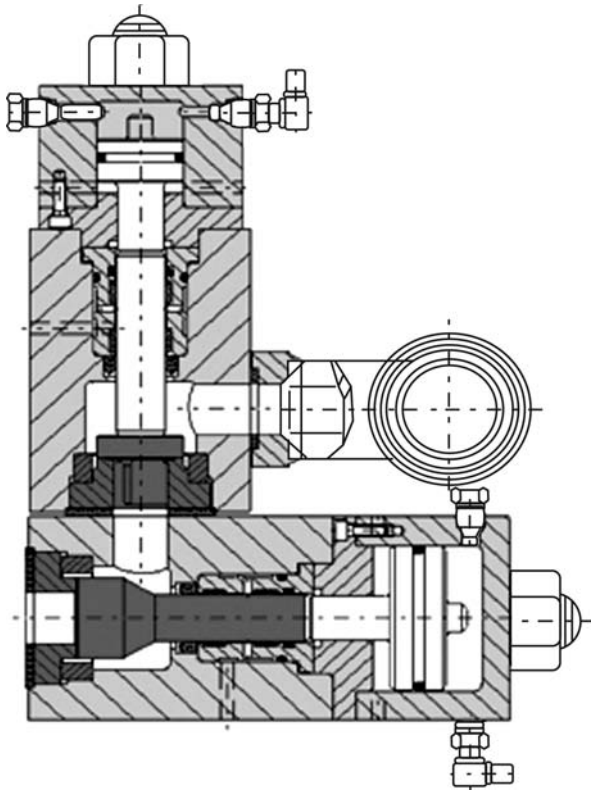

Figure 32.4 *Two-stage homogenizer* (*used with kind permission from Invensys APV*).

at higher pressures due to increased clustering of globules. The resultant cream will be more viscous, which may be a desirable outcome for particular types of cream products.

Higher homogenization pressure will increase the overall stability of fat globules as smaller fat globules are less likely to aggregate. The application of heat will have the opposite effect; higher pressure plus sufficient heat treatment will decrease stability (see Section 32.4.2.2). The different effects of heating and homogenization, and combinations of these two processes, must clearly be differentiated.

32.4.3.3 Lipolysis. Cream contains around 1–2 mg per liter of milk of a native milk lipase (Weihrauch 1988). The pH optimum of this enzyme is 8.5, and there is sufficient present in milk to cause rancidity within a few minutes. It is highly selective for hydrolysis of butyric acid, found primarily at the sn-3 position of triacylglycerides. The enzyme is inactivated by holding cream at 78°C for 10 s, whereas under pasteurization conditions at 72°C for 15 s, 10–15% of the original amount of lipase remains active. The native milk lipase is mainly associated with casein micelles, although there is an increasing proportion adsorbed onto fat globule surfaces at lower temperatures. This explains in part why lipolysis of raw milk is more rapid at a cooler temperature of 15°C. Lipolysis resulting in free fatty acids occurs at a faster rate in raw milk held at 4°C for 24 h compared to holding at 8°C (Wiking and others 2002).

Lipase from psychrotrophic bacteria is more heat stable than native milk lipase, and adsorbs onto the fat globule surface. Pseudomonas bacteria produce a phospholipase

that acts upon the MFGM, allowing lipase access to the inner triacylglyceride core. The phospholipase activity in skim milk is not eliminated completely by pasteurization (Koka and Weimer 2001). The affect of psychrotrophic bacteria on the quality of dairy products has been reviewed by Champagne and others (1994), and by Meer and others (1991).

The rate of lipolysis can be reduced by holding cream at a lower pH of around 6.7, compartmentalization of the lipase by association with the casein micelle, protection of the lipid phase by the MFGM layer, and possibly by proteose-peptone 3 inhibition. Lipolysis is more common in late lactation and at a higher frequency of milking. Spontaneous lipolysis occurs in raw milk with a high level of lipoprotein lipase associated with fat globules (Bachman and Wilcox 1990). Prevention of lipolysis, and the growth of psychrotrophic organisms can be achieved by addition of hydrogen peroxide (Ozer and others 2000) with subsequent addition of catalase to remove the hydrogen peroxide. This treatment has efficacy in parts of the world where refrigeration is not readily available. Hydrogen peroxide treatment of milk for cheddar and Swiss cheese manufacture is permitted in the United States; however, it should be noted that this type of treatment of milk may not be permitted in some countries.

The natural MFGM layer protects the lipid phase in cream from hydrolysis by the lipase. MFGM reduces surface tension to low values of less than 2 mN/m. The native lipase cannot displace the MFGM components and adsorb onto the surface because of this already low surface tension; therefore, lipolysis is hindered.

Homogenization creates extra fat globule surface area, which is coated primarily with serum proteins. These are mainly casein micelles and casein micellar fragments rather than whey proteins (Gelin and others 1994). The surface tension is raised to around 15 mN/m and this increases the propensity for the displacement of the newly formed membrane material by the lipase. Once the lipases have adsorbed to the surface, access to the lipid interior of the globule is enhanced. For this reason, milk is first pasteurized to inactivate the native milk lipase before homogenization. It has been noted, however, that homogenization of ultra high temperature (UHT) treated cream with added lipases has increased the susceptibility to lipolysis compared to raw cream or pasteurized cream (Iametti and others 1997).

32.5 STABILITY

Stability is often an ill-defined term used in the scientific and technological literature. When considering fat globules, stability may mean the propensity for

1. Rupture of the membrane coating to form pools of free fat;
2. Clustering of fat globules during homogenization to form an aggregate structure that traps the serum phase;
3. Heat-induced aggregation;
4. The feathering phenomenon found after cream is added to hot coffee;
5. Clumping of fat globules at the air–water interface, such as is required for ice-cream manufacture;
6. Gelation of proteins that settle to the bottom of UHT-treated products;
7. Displacement of the natural MFGM layer with surfactants to induce aggregation of globules;

8. Aggregation of globules to form a cream layer; or
9. Rupture of the MFGM of two adjoining globules that subsequently combine to form either a single larger globule or a partially joined dumb-bell-shaped structure.

Each of these mechanisms will impose different physical properties onto a cream product.

After some initial consideration, stability is intuitively a desirable outcome. However, in some instances, instability may be required to process milk into a particular dairy product. Two such products containing air cells are ice-cream and whipped cream, where fat globule clumping is necessary. An obvious example where instability is required is large-scale phase inversion of cream to form butter. The type of stability must be stated when discussing emulsions, such as dairy cream. To make this discussion clearer, different terms are used to indicate different types of instability.

32.5.1 Rupture

Rupture is the process where part of the MFGM layer is broken and removed from the globule surface. Breakage of the MFGM layer is unlikely to take place during laminar shearing of cream as the shear forces are not sufficiently high (see Section 32.6.1). Turbulent flow may, however, result in membrane rupture. Adsorption of globules to an air–water interface may allow fat crystals to pierce the air cells and spread liquid oil over the interface. Heating will promote coalescence and loss of membrane material into the serum phase. An example of heat-induced rupture is the formation of pools of fat in Mozzarella cheese (Rowney and others 2003). Cooling can also result in partial loss of MFGM material.

32.5.2 Clustering

Clustering is used to describe aggregation of fat globules induced by heat, homogenization, or both processes. Higher shear rates will increase the rate of globule collision and, as a consequence, the degree of clustering. A higher fat content and larger fat globules will result in more collisions and more clustering. Beating of air into cream will facilitate adsorption of globules at the air–water interface, and therefore the extent of clustering. Replacement of native MFGM by caseins as a consequence of homogenization will facilitate bridging and, therefore, clustering of adjacent fat globules by adsorbed casein micelles.

32.5.3 Clumping

Clumping occurs when globules aggregate following some change to the surface characteristics, such as addition of surfactants in the ice-cream manufacturing process. Clumping of native fat globules is hindered due to the net negative charge on the surface and the presence of a steric hindrance layer of glycoproteins. Addition of nonionic surfactants, such as those of the Tween series or monoacylglycerides, may displace caseins from the surface of homogenized fat globules and decrease stability to aggregation. This is not a heat-induced effect. It can be employed to partially destabilize fat globules in ice-cream manufacture (Goff 1997). Surfactants will decrease cluster formation during homogenization and increase clumping of fat globules that form during the whipping process.

32.5.4 Coalescence

Coalescence refers to the physical joining of two globules, either completely or partially, following rupture. In this instance fat crystals may be shared by two joined globules, a

mechanism that takes place in butter manufacture. Crystals of fat within the fat globules may pierce the MFGM layer if the crystals are correctly orientated, and join together two or more adjacent fat globules. This will most likely only happen under conditions where fat globules are under shear or turbulent conditions.

Coalescence requires some solid fat to be present. At lower temperatures where the fat is mostly solid, crystals may not be as free to orientate and pierce another fat globule. At higher temperatures there may not be sufficient crystals available to link globules, and coalescence under shear conditions will result in the formation of larger spherical globules. Some liquid fat is still required to allow the globules to initially bond together. This property can be exploited to increase the viscosity of cream by homogenization while the fat is partially solidified. Raising the temperature will melt the fat crystals and reduce partial coalescence, and therefore reduce viscosity. Temperature fluctuations from 5°C to 30°C followed by cooling may result in coalescence (see Section 32.6.3).

Coalescence of fat globules will not, of course, decrease the volume or the weight of fat in cream, but it will result in a decrease in total interfacial surface area due to an increase in average globule size. One consequence of this is the release of MFGM material, the main solid component of buttermilk, into the serum phase.

32.5.5 Creaming

Creaming refers to the rising of fat globules to the surface of milk or cream. This property is exploited to produce cream from milk by centrifugal action. The rate of creaming increases for larger fat globules, for higher fat content, and under conditions where the agglutinin reaction is facilitated (see Section 32.6.4). Creaming rate can potentially increase as a consequence of heat treatment or homogenization. Carrageenan can be added to increase viscosity, which slows down the rate of creaming during storage.

Fat globules rise at a velocity v, due to a density difference with the serum phase. This is given by the Stokes equation,

$$v = \frac{2g(\rho_s - \rho_g)r^2}{9\eta_s}, \tag{1}$$

where r is the radius of the globule, ρ_s is the density of the serum phase, ρ_g is the density of the fat globule, and η_s is the viscosity of the serum phase. The most temperature-dependent of these parameters is viscosity; at higher temperatures the viscosity is reduced and the rate of creaming increases. Homogenization of milk fat with polyoxyethylene-4-sorbitan monolaurate (Tween 21) with subsequent UHT treatment results in less creaming compared to treatment with other emulsifiers such as sorbitan tristearate (Span 65), sorbitan monopalmitate (Span 40), or 90% monoacylglyceride preparation from palm oil (Mayhill and Newstead 1992).

32.6 PHYSICAL PROPERTIES

32.6.1 Rheological Properties

Cream with a fat content more than about 40% exhibits a pseudoplastic (or shear thinning) flow profile with a yield stress. This type of flow profile is characterized by a decreasing apparent viscosity as the rate of shearing increases. Below this fat level the flow profile

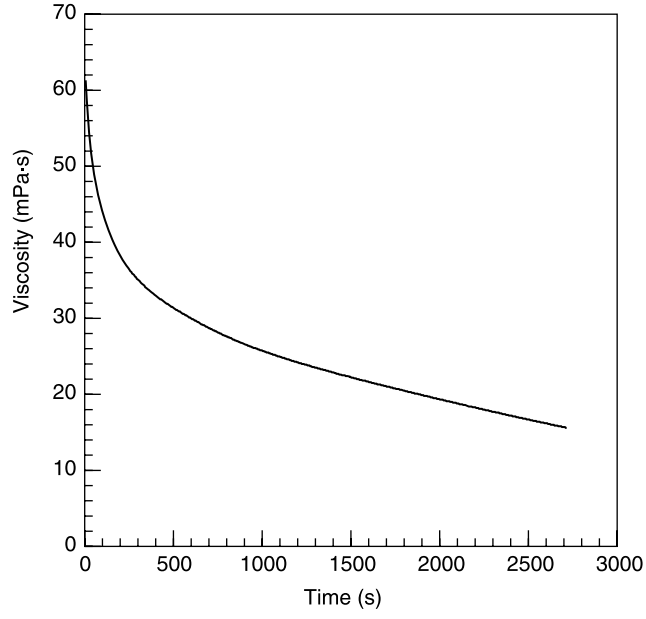

Figure 32.5 *Viscosity of homogenized 30% fat cream under shear conditions of 1465 s^{-1} over a period of 45 min at 31°C. Cream was homogenized in a one-stage homogenizer (Type 8.30H, APV – Rannie Inc., St. Paul, Minnesota) at pH 7, 50°C, and 20.0 MPa.*

tends to be Newtonian, where shear stress is proportional to shear rate. The presence of a yield stress means that no flow can occur until a stress has been applied that exceeds this characteristic stress. Cream also exhibits thixotropic time-dependent behavior where viscosity decreases slowly over time at a fixed shear rate (Figs 32.5 and 32.6). Both of these phenomena occur due to aggregation of fat globules to form flocs that trap the serum phase. At higher shear rates or over a period of constant shearing, these flocs break apart, concomitant with less serum phase trapped within the floc structure, and the viscosity is reduced. The re-bodying effect results in cream with a plastic flow behavior, characterized by a linear shear-stress–shear-rate profile with a relatively large Bingham yield stress, corresponding to the intersection of the linear flow profile with the stress axis. Bingham yield differs from yield stress in that the former is an extrapolation of a linear part of the flow profile to the stress axis, whereas the latter is an extension of any shaped flow curve to the stress axis by measuring stress at increasingly lower shear rates. The experimentally measured yield stress is a closer representation of actual yield stress than Bingham yield.

Flocs that trap serum behave essentially as large particles. This increases the effective volume fraction of particulate matter in the cream, and viscosity increases according to the Kreiger–Dougherty equation,

$$\eta = \eta_0 \left(1 - \frac{\phi}{p}\right)^{-[\eta]\phi} \tag{2}$$

where η is the viscosity of the cream, η_0 is the viscosity of the continuous aqueous phase, $[\eta]$ is the intrinsic viscosity (related to the shape and the concentration of fat globules),

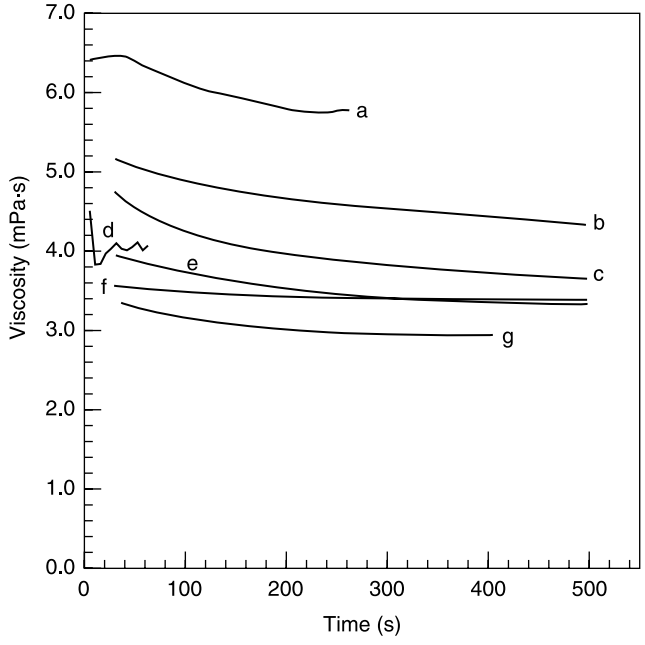

Figure 32.6 *Viscosity of 30% fat (unhomogenized) cream and recombined anhydrous milk fat emulsions prepared by homogenization under shear conditions of 1465 s^{-1} at 31°C. Recombined emulsions were prepared using a one-stage homogenizer (Type 8.30H, APV–Rannie Inc., St. Paul, Minnesota) at pH 7, 50°C, and 20.0 MPa. (a) Unhomogenized cream; Recombined creams containing globules coated with (b) β-casein, (c) α_{s2}-casein, (d) Tween 80, (e) κ-casein, (f) phosphatidylcholine, and (g) α_{s1}-casein.*

ϕ is the volume fraction (the fraction of the total volume occupied by fat globules), and p is the volume fraction at closest pack (approximately 0.72).

Addition of polysaccharide stabilizers to cream increases the viscosity of the aqueous continuous phase and slows down the rate of collisions between fat globules. The effect is to slow down the rate of creaming. Addition of stabilizers such as alginate or carrageenan can also be used to increase the viscosity of dessert creams.

Homogenized 30% fat cream has a viscosity of around 60 mPa·s at 31°C, which decreases slowly under shear conditions of 1465 s^{-1} to around 15 mPa·s after 45 min, and continues to decrease thereafter (Fig. 32.5). The viscosity of 30% fat unhomogenized cream is approximately 6.4 mPa·s at 31°C and this decreases to a much lesser extent to around 5.8 mPa·s after 5 min under the same shearing conditions (Fig. 32.6). Emulsions containing milk fat globules coated with a nonionic Tween 80 surfactant, phosphatidylcholine, or individual caseins show similar behavior to unhomogenized cream with about the same magnitude of viscosity. Cream and prepared emulsions thus exhibit time-dependent thixotropic behavior. The process of homogenization yields a cream with a higher viscosity and increases the time taken to break apart clusters of fat globules.

Unhomogenized cream has a very small yield stress of less than 0.2 Pa. Homogenization increases the yield stress to around 0.6 Pa. Coating fat globules with milk proteins such as whey or caseins, or surfactants such as Tween 80 or phosphatidylcholine reduces the yield stress to a value approaching zero. These recombined globules contain very thin (perhaps monolayer) coverage of membrane material. With recombined milk fat emulsions, viscosity is lowered to around 3–5 mPa·s and the extent of time-dependent

viscosity reduction is lessened, despite the use of homogenization to prepare the emulsions. This indicates that non-native fat globule membranes do not interact and form clusters to the extent of native globules or homogenized globules coated with casein micelles.

The pressure at the interface of an emulsion globule is given by the Laplace pressure P, where

$$P = \frac{2\gamma}{r}. \tag{3}$$

With a surface tension γ of 1.5 mN/m and a globule radius r of 10^{-6} m, the Laplace pressure is 3000 Pa. Under laminar shearing conditions, the shear stress τ, in units of Pascals, is given by

$$\tau = \eta \frac{\mathrm{d}\gamma}{\mathrm{d}t} \tag{4}$$

where η is the viscosity (about 6 mPa·s for 30% unhomogenized fat cream at 31°C) and $\mathrm{d}\gamma/\mathrm{d}t$ is the rate of shearing, in units of s^{-1}. For globules to be broken apart under laminar shearing conditions the shear stress must be higher than the Laplace pressure. For this to occur the shear rate will need to exceed $5 \times 10^5\ s^{-1}$ for globules of radius 10^{-6} m. This high shear rate is unlikely to be exceeded under most laminar shear processing conditions. Rapid shearing is therefore not likely to rupture fat globules, and this likelihood will only decrease if globules are made smaller by homogenization. Beating of air into cream will, however, disrupt fat globules by the forces associated with adsorption to the air–water interface.

Viscosity of cream rises during refrigerated storage. Cream separation at 30°C results in a higher viscosity compared to cream separated at 45°C. A possible explanation for these observations may be formation of triacylglyceride crystals (to a greater extent at the lower temperature), which pierce the MFGM layer and link adjacent fat globules through partial coalescence.

32.6.2 Effect of Cooling

Cooling of fat globules leads to a loss of MFGM material from the surface. This is an irreversible process, and is countered by reversible adsorption of a class of proteins called cryoglobulins. These proteins facilitate the agglutinin creaming reaction. At low temperatures triacylglycerides form a network structure of crystals within the globule, providing some rigidity.

Frozen cream results in large-scale phase separation upon thawing. This is not a desirable attribute for consumer cream, but it can still be used as a food ingredient to standardize homogenized consumer milk to a higher fat content, or in butter manufacture. If cream is to be frozen, it must be done quickly to ensure that large ice crystals do not form that pierce the native MFGM layer and induce fat globule coalescence. Rapid freezing can be achieved by immersion into liquid nitrogen, yielding smaller ice crystals. The integrity of the MFGM layer is critical for the maintenance of freeze–thaw stability. Cycling of temperature during frozen storage can have a detrimental effect on cream stability.

32.6.3 Re-Bodying Effect

In cream with more than 25% fat, temperature fluctuations can result in an increase in viscosity. This phenomenon is called the re-bodying effect (Walstra and others 1999).

Holding cream at 5°C for several hours, followed by warming to 30°C and holding for 30 min, then slow cooling again, produces clusters of fat globules. This may result in formation of a gel under some conditions. The slow cooling back to 5°C allows crystals of triacylglycerides to form that bridge adjacent globules. Re-bodying is eliminated by shearing, but this is a reversible process that occurs again once the cream is cooled.

Re-bodied cream exhibits more leakage of the serum phase from the fat globule network (syneresis). This phenomenon occurs with washed cream but not with recombined cream. The presence of phosphatidylcholine in the membrane layer may be a necessary criterion for re-bodying. The re-bodying process can be utilized to improve the whipping properties of UHT treated cream.

32.6.4 Agglutination

At cold temperatures cryoglobulins (lipoproteins and immunoglobulins) adsorb onto the surface of bovine fat globules. This facilitates the agglutinin reaction where fat globules aggregate, trapping serum phase in the globule network structure, and increasing the rate of creaming. The problem is not as great above 37°C, where cryoglobulins do not adsorb to the surface of globules. The agglutinin reaction does not occur significantly with goat or sheep milk, which may partly explain why cream, and hence butter, is less common from these two types of milk compared to bovine milk. The lower pressure second stage of homogenization is commonly used to break up agglutinin-induced clusters to prevent creaming.

Heat treatment of cream at 71°C for 20 s has no effect on minimizing the agglutinin reaction. Treatment at 65°C/30 min, 73°C/20 s, and 78°C/20 s reduces creaming by 10%, 25%, and 100% respectively (Walstra and others 1999), possibly due to immunoglobulin denaturation. If warm milk is separated, the agglutinin factors remain in the skim phase. Separation of cold milk results in the factors locating in the cream phase. Mixing the cold skim with the warm cream will reduce the agglutinin reaction and subsequent rate of creaming.

The agglutinin reaction is more pronounced in colostrum and less so in late lactation milk. Agitating milk at low temperatures reduces creaming, possibly by inducing aggregation of agglutinin factors.

32.7 TYPES OF CREAM

The fat content of cream can vary from values above that of milk (a requirement to distinguish it from milk) to the maximum amount that can be held in an emulsion, beyond which cream phase-inverts to become butter. The maximum fat content in cream is typically around 70–80%, and the propensity for possible subsequent phase inversion will depend upon the shearing conditions imposed during the concentration process. More turbulent conditions will produce butter more readily, thus lowering the maximum amount of fat that the cream can hold as a fat-in-water emulsion.

The extent of separation of milk to form cream dictates the cream classification. "Half and half" cream contains the lowest typical fat content of all types of creams, usually around 10–12%. It is commonly served as a whitener for hot tea and coffee. Light cream is usually around 20% fat, and heavy cream about 40% fat. Cream containing around 48% fat is referred to as double cream. Cream can be heated to form fat globule clusters and to also denature whey proteins. This type of cream with around 55% fat is called clotted cream, and is more common in Britain. Cream with the maximum amount of fat, whilst maintaining a fat-in-water emulsion, is termed plastic fat.

Cream with a fat content as high as 80% can be dried to a powder. This requires the addition of surface-active proteins such as sodium caseinate to maintain the encapsulation properties of the dried powder particle. The cream must first be heat treated to minimize lipolysis during storage. Antioxidants can be added to prevent oxidation of the milk fat.

32.7.1 Whipping Cream

The steps involved in whipping cream preparation are shown in the flow diagram in Figure 32.7, adapted from Walstra and others (1999). The amount of air incorporated into the whipped cream structure is given by the over-run. This is expressed as a percentage,

$$\frac{V_f - V_i}{V_i} \times 100, \tag{5}$$

where V_f is the final whip volume containing air and V_i is the initial volume of the unwhipped cream.

Whipping cream is available as a pasteurized product containing 35–40% fat, with a minimum of 30% fat required before successful whipping can occur. The process of pasteurization limits the growth of psychrotrophs, which produce heat-stable lipases. *Bacillus cereus*, which causes fat globules to become unstable, can be reduced by additional heat treatment at 85°C for 30 min. *Bacillus cereus* has also been implicated in curdling of sweet whipped cream (Buck and others 1992). Other types of heat treatment include

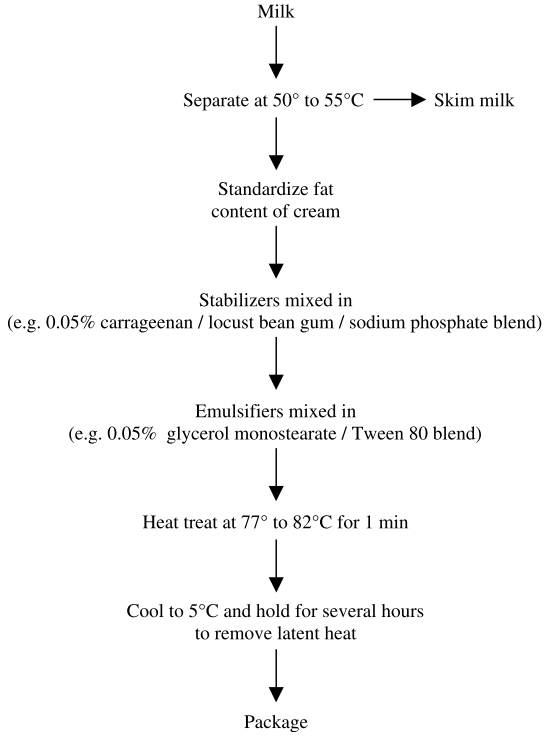

Figure 32.7 *Preparation of whipping cream.*

use of plate heat exchangers at greater than 100°C, or in-bottle treatment at 103°C for 20 min. Deodorization to remove off-aromas can take place in an open vessel with stirring.

Whip volume depends upon the fat content of the cream and the thickness of adsorbed fat globules at the air–water interface (van Aken 2001). Whipped cream contains air cells of 10–100 μm in size. Soluble surface-active proteins (β-casein and whey proteins) adsorb to the air–water interface during the initial stages of whipping and form an air cell network (Besner and Kessler 1998a). Fat globule aggregates adsorb to the interface and provide some stability and rigidity to the maintenance of the air cells. This is a two-step process of complete coalescence of smaller globules followed by partial coalescence of larger globules (Smith and others 2000). Harder milk fat leads to better whipping properties (Overbeck and others 1994), and for this reason, the best whip structure is obtained at 10°C. Whipping cream may require several hours of tempering at this low temperature to initiate sufficient fat crystallization to promote fat globule coalescence. The globules leak some liquid oil, which spreads across the interface, providing a sticky surface that catches fat globules at the interface. Excessive whipping causes the fat globule aggregates to become so large that they cannot stabilize the air cells. These large aggregates form butter granules, and ultimately butter if excessive whipping continues. Adsorption of an excessive amount of fat crystals at the air–water interface results in cream with long whipping time and low over-run (Brooker 1990). Higher amounts of fat will decrease the whipping time and the over-run, increase the firmness of the whipped cream, and decrease the amount of serum leakage from the network of air cells.

Homogenizing cream reduces the propensity for whipping (Besner and Kessler 1998b), possibly due to the fat globules being too small to form a cohesive aggregate structure at the air–water interface, or perhaps the newly created casein-coated globule surface prevents fat crystals from piercing through the membrane. In this case, whipping time will increase and the firmness of the whipped cream will be reduced. However, at low homogenization pressure, coalescence is promoted. This is because the globules are larger and do not contain as much casein at the globule surface, showing clearly that the surface characteristics are important in dictating whipping functionality.

Addition of emulsifiers such as Tweens or monoacylglycerides decreases whipping time, increases whip firmness, and decreases serum phase leakage from the air cells. This is further evidence for the importance of the fat globule surface layer in forming a whipped cream structure. Comparisons of the effect of different emulsifiers on whipping cream functionality is provided by Towler and Stevenson (1988).

Carrageenan can be added to minimize leakage of serum phase from the air cell network (Stanley and others 1996). Stabilizers can also increase whipping time and reduce over-run (Camacho and others 1998). Synergistically with emulsifiers, stabilizers also reduce the viscosity of UHT-treated processed whipping cream during storage (Towler 1988).

Whipping cream can be made available in a pressurized can that provides the necessary air to create a structure with sufficient over-run to provide for a firm product. Over time, the larger air cells grow by adsorbing smaller cells, a process called Ostwald ripening, which culminates in foam collapse. Whipped dairy topping is another product packaged in aerosol containers. A typical formulation consists of 30% milk fat, sucrose for flavoring, sodium caseinate, corn syrup solids, stabilizers, and emulsifiers.

Although fat globules stabilize a foam network, fat can also depress foam formation. The apparent contradiction is resolved by realizing that fatty acids produced by lipolysis of triacylglyceride fat reduce foam volume. Spreading of intact and unemulsified triacylglyceride fats will also depress foam formation.

Churning of cream to form butter is a form of flotation, where air cells pick up fat globules and form butter granules at the air–water interface. As smaller globules do not adsorb as well as the larger ones, churning has the effect of reducing the average fat globule size. Churning or beating at a low temperature (around 5°C), where liquid fat is not sufficient, produces whipped cream rather than butter as there is not sufficient liquid fat at the air–water interface to catch fat globules to subsequently form butter granules.

Cholesterol can be removed from cream by complexation with β-cyclodextrin. The functional properties of whipped cream after using this process are not significantly different from unprocessed whipped cream (Shim and others 2003).

32.7.2 Sterilized Cream

The steps involved in sterilized cream preparation are shown in the flow diagram in Figure 32.8, adapted from Walstra and others (1999). As the sterilization heat treatment comes after the homogenization step, the likelihood of heat-induced aggregation is increased.

The sterilization process minimizes fat lipolysis; however, the Maillard browning reaction can occur during storage. Sterilized cream should be homogenized to prevent oiling-off (phase separation). Sodium alginate can be added to increase viscosity and prevent precipitation of proteins. Sterilized cream can be sold as "half and half", or coffee cream containing around 25% fat.

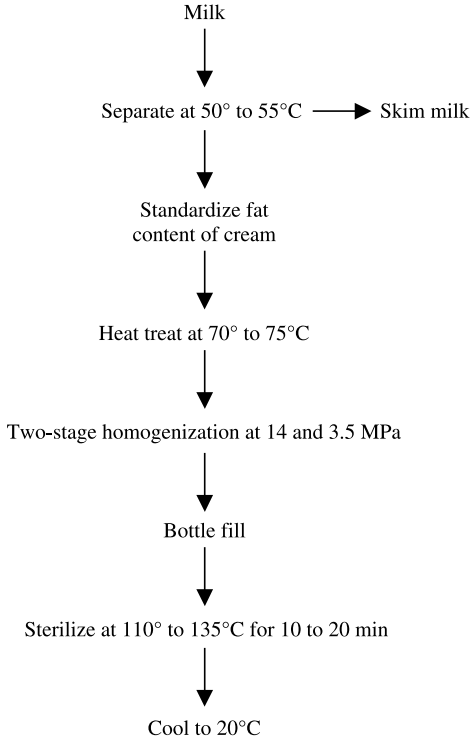

Figure 32.8 *Preparation of sterilized cream.*

32.7.3 UHT Processed Cream

UHT milk has a cooked flavor due to the high-temperature heat treatment. The steps involved in the preparation of UHT-treated cream are shown in the flow diagram in Figure 32.9, adapted from Walstra and others (1999). In some processes homogenization can take place before the UHT step, after the UHT step, or split such that the first homogenization stage is applied before, and the second stage after the UHT treatment step.

The UHT treatment process can result in feathering of cream when added to hot beverages such as coffee. The feathering process is caused by aggregation of fat globules coated with casein micelles from the homogenization process. The extent of feathering depends upon the pH, concentration of calcium ions, and the temperature of the beverage. A film comprised of milk constituents, primarily aggregated fat globules, can form on the surface of hot tea (Spiro and Chong 1997). This is exacerbated by higher calcium and lower concentration of tea polyphenols in solution, and is more pronounced for UHT treated milk. Sequestering of calcium will help in eliminating feathering.

UHT-treated cream is susceptible to age gelation, which is manifested as a thickening of the cream during storage. Homogenization takes place aseptically after UHT heat treatment to prevent heat-induced coagulation of proteins and clustering of fat globules. Creaming and clustering of fat globules can be reduced by addition of stabilizers. There is some suggestion that the cream microstructure of UHT-treated milk does not depend on homogenization being performed before or after heat treatment (Hillbrick and others

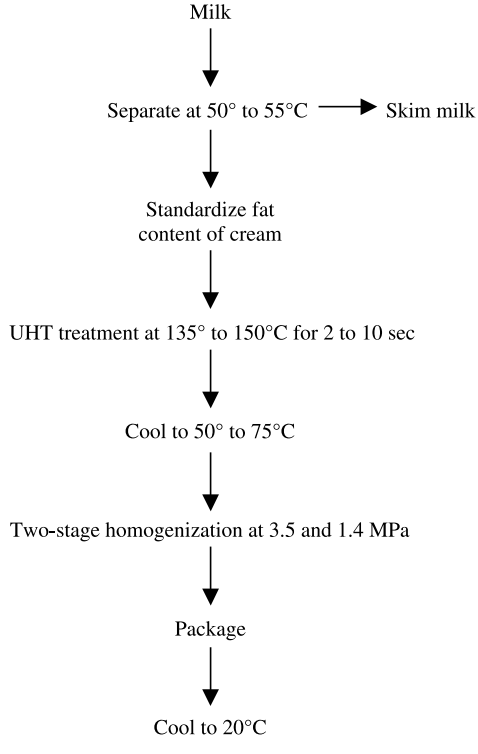

Figure 32.9 *Preparation of UHT-treated cream.*

1999). This may allow for homogenization to take place before heat treatment, with the elimination of aseptic homogenization during processing.

Inadequate homogenization can result in creaming during storage of UHT-treated milk, limiting the shelf-life of this product (Hillbrick and others 1998). High-pressure processing of UHT-treated cream at the very high pressure of 450 MPa can cause fat globule aggregation and increase viscosity through the formation of an extensive network of fat crystals (Dumay and others 1996).

UHT-treated cream has a shelf-life of months if stored at around 5°C. Storage at higher temperatures can exacerbate fat globule agglomeration and creaming.

32.7.4 Recombined Cream

Recombined cream is produced by emulsifying milk fat with surfactants such as milk proteins or buttermilk solids. Filled cream contains other nondairy sources of fat, and will not be discussed in this chapter.

The nature of the membrane layer on the surface of globules will have some impact upon crystal formation within the globule. Buttermilk, serum phase from anhydrous milk fat manufacture, or other similar emulsifiers are necessary to maintain good whipping properties of recombined cream. In these recombined creams, an interface containing adsorbed caseins will result in a lower temperature to induce crystallization and less fat globule coalescence in a whipped formulation compared to globules coated with whey proteins (Relkin and others 2003). Whey protein isolate and sodium caseinate produce a more stable butteroil emulsion compared to skim milk proteins (Segall and Goff 1999). Recombined cream formed by emulsifying a medium melting point (dropping point of 26°C) fraction of butteroil with buttermilk results in a cream with less cholesterol compared to natural cream (Scott and others 2003). UHT processing of recombined milk can promote creaming. A lower melting point milk fat fraction (dropping point of 23°C) results in more rapid creaming over a two-month period compared to a higher melting fraction (Mayhill and Newstead 1992).

32.7.5 Cream Liqueur

The basic principles involved in the manufacture of cream liqueurs are illustrated in the flow diagram in Figure 32.10, adapted from Banks and others (1982). The final composition of a cream liqueur is 14% ethanol and 40% solids. Ionic conditions, pH, and the type of caseinate will affect the structure of cream liqueurs. A reduction in pH below 6.4 results in increasing flocculation of fat globules (Dickinson and others 1989). Sodium chloride will increase the viscosity of sodium-caseinate-stabilized emulsions in cream liqueurs (Lynch and Mulvihill 1997). Addition of cream liqueur to coffee will mix into the beverage rather than form a cream layer above the coffee. By adding trisodium citrate to sequester calcium, and at a high fat content of 40%, a stable cream liqueur can be produced that floats on top of hot coffee (Muir 1988).

32.8 VOLATILE COMPONENTS

Cream contains many desirable volatile components that contribute to flavor. For a detailed analysis on the volatile components found in cream and different cream fractions using gas chromatography, see Wong and Patton (1962) and Siek and Lindsay (1968).

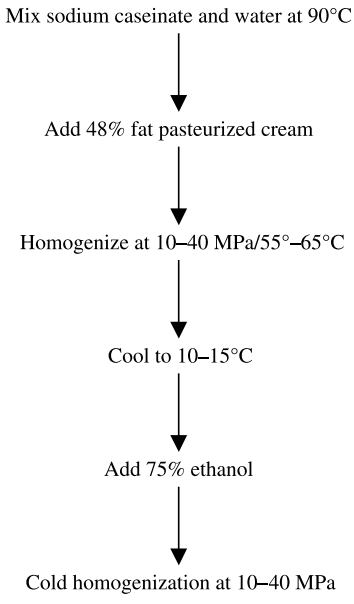

Figure 32.10 *Preparation of cream liqueur.*

32.8.1 Off-Flavors

Vacreation is a steam distillation process used to remove off-flavors and odors from cream. These undesirable components derive from the type of pasture eaten by the cows. In the vacreation process, steam is injected into cream and flash evaporated under reduced pressure. The undesirable volatiles are removed with the steam. This process has the potential to heat-induce coalescence of fat globules, and also to partially homogenize the larger globules through the turbulent action of the steam injection process. A more detailed description can be found in Towler (1994).

An alternative to vacreation, spinning cone technology, can be used to remove off-flavors and odors from fluid products under reduced pressure (Fig. 32.11). This apparatus consists of a series of alternating stationary and rotating concentric cones. Fluid is injected into the top of the column, flowing down the first stationary cone and onto the second cone, which is rotating. The fluid moves upwards and outwards as a thin film and onto the next cone, which is stationary (Fig. 32.12). This process is repeated as the fluid moves down the series of cones. The cones serve the purpose of increasing the total surface area of the injected fluid, hence increasing the efficiency of separation. At the same time, steam is injected into the bottom of the column and moves towards the top, stripping volatiles from the injected fluid product. Deodorized fluid is removed from the bottom of the stack of cones. The volatiles in the steam phase can later be retrieved by condensation.

32.9 SUMMARY

The fat in cream is emulsified in an intricate encapsulated package containing multilayers of enzymes, other proteins, and lipids that participate in these reactions. Despite the apparent simplicity of cream as a processed dairy product, there is an immense and hidden

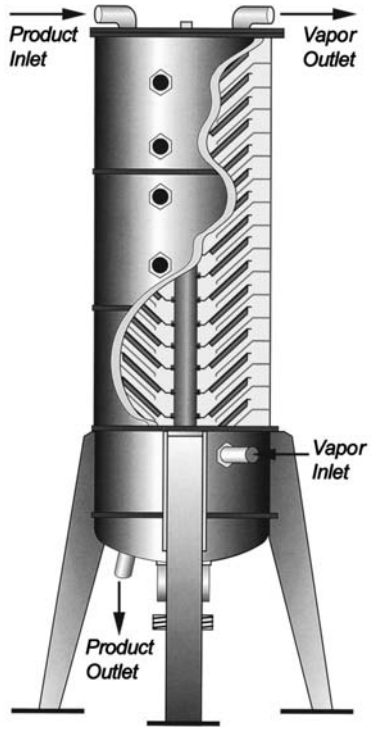

Figure 32.11 *Spinning cone device* (*used with kind permission from Flavourtech*).

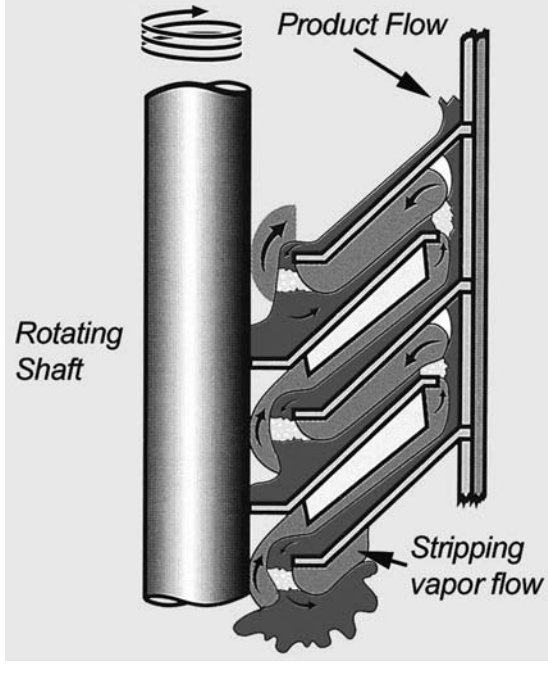

Figure 32.12 *Spinning cone internal configuration* (*used with kind permission from Flavourtech*).

complexity within this food. The many different components of cream interact and produce desirable flavor and texture through chemical and physical reactions. Processing conditions such as heat and shear flow induce other reactions that alter the physical stability of cream as an emulsion. These can be employed to produce many different types of cream with different flavor and textural attributes for use as food ingredients.

ACKNOWLEDGMENTS

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33

Influence of Processing on Virgin Olive Oil Quality

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33.1 Introduction 752
33.2 Process of Extraction of Virgin Olive Oil 753
33.2.1 Preliminary Operations 754
33.2.2 Paste Preparation 755
33.2.3 Liquid–Solid Separation 756
33.2.4 Liquid–Liquid Separation 757
33.2.5 Storage 758
33.3 Influence of Processing on Main Characteristics of Virgin Olive Oil 758
33.3.1 Volatile Compounds 758
33.3.2 Phenolic Compounds 759
33.3.3 Tocopherols 761
33.3.4 Chlorophylls and Carotenoids 761
33.4 Quality Markers of Olive Oil 762
33.4.1 Regulated 763
33.4.2 Chlorophylls and Carotenoids 765
33.5 Traceability Markers of Olive Oil Processing 765
33.5.1 Phenols and Volatiles 765
33.5.2 Chlorophyll and Carotenoid Indexes 766
References 767

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

33.1 INTRODUCTION

The olive tree, *Olea europaea* L., is the best known and most widely spread species of the Oleáceas family, its cultivation extending mainly to countries in the Mediterranean area. The tree blooms in May with small white flowers and, almost immediately after, in May or June, the fruit begin to form, culminating in maturation towards the end of autumn.

Its cultivation began more than 6000 years ago, making it one of the oldest cultivated plants in the Middle East, and spread west on both sides of the Mediterranean Sea. It reached Spanish shores with the Roman Empire, but it was the Arabs who promoted cultivation in Andalucia, making Spain the prime producing country in the world. The olive tree was brought along from the Mediterranean basin with the discovery of America in 1492. From Seville, the first olive trees were taken to the Antilles, and then to the continent. By 1560, olive groves were productive in Mexico and later in Peru, Chile, Argentina, and the state of California in the United States. In more recent times, olive growing has continued to expand to places far from its origin to southern Africa, China, Japan, and Australia (Civantos 2004).

There are some 850 million olive trees in the world, occupying an approximate surface area of 8.7 million ha, of which 95% is concentrated in the countries of the Mediterranean basin. Spain is the leading exporter of olive products, with some 170 million olive trees in more than 2.2 million ha of groves, of which 60% are located in Andalucía. Other countries with substantial populations of olive trees are Italy (125 million), Greece (120 million), Turkey (83 million), Tunisia (55 million), Portugal (49 million), and Morocco (33 million).

The fruit of the olive tree, "oliva" or "aceituna", is defined as a drupe and so exhibits a growth curve of the sigmoidal-double type, describing three stages. In the first stage, the weight of the fruit increases remarkably, followed by a stage of weight maintenance, and, finally, a fast growth phase due mainly to an increase in cell volume. Each one of these stages shows a variable duration according to variety (Roca and Mínguez-Mosquera 2003).

The component parts of the olive are the epicarp (or epidermis), the mesocarp (or flesh), and the endocarp. The olive mesocarp, which is also referred to as pulp, accounts for 70–90% of the olive weight (wet basis); the endocarp (stone or pit) comprises 30–10%, and includes the seed (1–3% of the whole fruit).

Olives differ from all other drupes in chemical composition, with a relatively low concentration of sugars, but a high oil content and a characteristic strong bitter taste. Composition depends on factors such as cultivar, latitude, climate, state of ripeness, and so on. The main constituents of the pulp are water (60–75%) and lipids (oil) (10–25%). Of the total oil weight, 98–99% consists of a mixture of triglycerides, free fatty acids, waxes, mono- and diglycerides, sterol esters and terpene alcohols, and phospholipids.

The earliest olive growers of each zone selected wild olives (acebuche) from woodlands for their productivity, fruit size, oil content, and environmental adaptation. Vegetative propagation maintains the characteristics of the initially selected first varieties. The repetition of this procedure (spread of cultivars/hybridization, selection of descendance/cloning) was responsible for a great diversity of autochthonous cultivars, products of chance in all the olive-growing zones throughout the world (Barranco 2004).

Close to 1500 olive cultivars are cataloged throughout the world, although some are the same cultivar under a different denomination. Cultivated olive trees are classified into three categories according to the use given to their fruits: table olive processing, oil extraction, or both purposes, also called double or dual-use cultivars.

The main varieties/cultivars of olive are different in morphologic, agronomic and technological characteristics, such as fruit weight, resistance to biotic factors, fat yield, and

adaptability to mechanical harvesting and pitting. The principal diseases of olive trees that affect production are repilo, caused by the mushroom *Spilocaea oleagina*, verticilosis, produced by *Verticillium dahliae*, and tuberculosis, caused by the bacteria *Pseudomonas syringae* pv. *savastoni*.

Of the 262 olive cultivars grown in Spain, 24 are classified as main varieties, occupying a considerable geographic area or dominant in at least one region. Only four varieties make up 60% of the olives grown, and one, the *Picual* variety, with more than 700,000 ha cultivated, produces practically half of all Spanish olive oil. Based on cultivated surface, *Cornicabra*, *Hojiblanca*, and *Lechín de Sevilla* are important.

The *Frantoio* cultivar occupies a cultivated surface of 100,000 ha and can be considered as the prototype of the Italian cultivars to produce an olive oil of good quality. *Koroneiki* is the main cultivar for oil production in Greece and occupies 50–60% of its cultivated surface. *Ayvalik* cultivar has a high percentage of oil and is considered as the Turkish variety for oil production because of its organoleptic quality. *Chemlali of Sfak* is the variety most important in Tunisia and represents 60% of the cultivated surface. The main Portuguese cultivar is *Galega Vulgar* or *Negrihna*, which represents about 80% of its olive production and is used primarily for oil production, although it is also appreciated as a Greek-style naturally black table olive. In Morocco, the *Picholine marocaine* cultivar occupies 96% of the cultivated surface and is a cultivar for dual use, as table olives and in oil production.

Of the annual world production of olive fruit – approximately 10 million tons – 10% is consumed as table olives, and the rest is processed for olive oil (Civantos 2004). The virgin olive oil is an excellent natural food obtained solely from fruits of the olive tree by mechanical or physical procedures like crushing, malaxing, centrifugation and decantation, and conditions, mainly thermal, that do not cause alteration of the oil. In these conditions, the olive oil has a very pleasant aroma and flavor, and can be used, without further treatment, for human consumption.

The excellent health properties (high monounsaturated fatty acid content, sufficient levels of polyunsaturated fats, antioxidants, and so on) of virgin olive oil are considered added values for the consumption of this oil. This has caused an increase in its market price. It is important for consumers to know the quality of the product and what they are paying for. This chapter focuses on the different studies made on the influences of processing conditions on certain components of virgin olive oil.

33.2 PROCESS OF EXTRACTION OF VIRGIN OLIVE OIL

The Spanish word *aceite* comes etymologically from the Arab word *az-zait*, meaning the juice of the olive. The oil obtained from olive fruits has served for centuries as a food, the prime material for lighting, a medicinal ointment, and a revitalizing liquid.

Today, olive oil is under great demand and has high profitability, thanks to the successful campaign that highlights its therapeutic and nutritive properties. It has an encouraging future and an increasing surface area of cultivation. Estimated world production for the 2005/2006 season by the International Olive Oil Council (IOOC) is 2,584,000 tons, with Spain (34.1%), Italy (23.2%), and Greece (16.4%) being the main producers.

Virgin olive oil has exceptional organoleptic characteristics and is practically the only vegetable oil that can be consumed raw, conserving intact its nutritive and functional properties. The deterioration in virgin olive oil is almost exclusively due to faulty handling of the fruit and of a poorly controlled production process. Independent of variety, only fruits

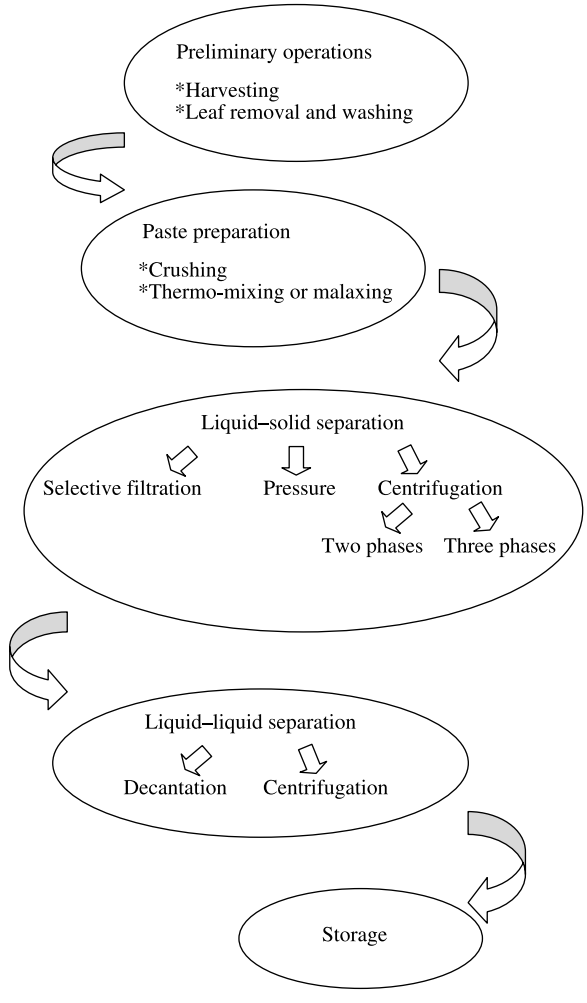

Figure 33.1 *Process of extraction of the virgin olive oil.*

that have suffered from the action of pests or diseases or have fallen on the ground before harvesting inevitably produce altered oil requiring refining due to its high free acidity and/or its disagreeable organoleptic characteristics. Figure 33.1 shows a general scheme of olive processing in order to obtain virgin olive oil (Alba 2004).

33.2.1 Preliminary Operations

The time of year at which the harvesting is carried out, as well as the system used, influences considerably the characteristics of the olive oil obtained. The fruit needs to be harvested at the optimum degree of ripeness, taking into account maximum oil content and best characteristics. The factors that have an impact on the optimum time of harvesting (Porras 1997), are:

- Resistance to the pull of the stalk of the olive;
- Oil content of the fruit;
- Changes in oil quality;

- Falling of the fruit; and
- Date of the previous harvest.

The resistance to stalk detachment is determined by the force necessary to break the pull of the fruit stalk, which can vary enormously throughout ripening and has a bearing on the performance of the operation if the harvest is manual. The harvest must coincide with the time that the green fruits disappear from the tree, which is also when the maximum oil content is reached.

The organoleptic characteristics of the oil deteriorate when the harvest of the fruit is delayed. If more fruity and aromatic oils are desirable, the harvest can be advanced by a few days. With a substantial percentage of green fruit, a better quality product is obtained, although the production yield of the oil is decreased. The natural fall of the fruit basically depends on the cultivar, although it can be influenced by climatic conditions and the health of the fruit. The end of the harvest should coincide with the time that the natural fall of the olive starts and would reach a percentage that would have a significant effect on the cost of harvest. A delay in the time of harvest also produces certain inhibition in floral differentiation of leaf buds, which can result in harvest losses the following year. Harvesting that spoils the fruit and produces damage, such as bruises, broken branches, or twigs, should be avoided.

In general, the fruit is brought down manually from the tree, known as hand picking, or mechanically, using a trunk shaker. The fruit is collected in cloth nets or plastic material that extends below the olive trees, occupying an area larger than the drop zone of the tree. Finally, the contents of these nets are poured into boxes or trailers for transferring to the olive-oil mill.

The daily capacity of oil extraction in the mill is normally lower than the rate of receiving the harvested fruit, requiring fruit to be stored in the so-called *trojes* (or silos) during a variable period of time. During this time, the olives can undergo various changes such as fermentation, enzymatic and microbial lipolysis, auto-oxidation of fatty acids, and so on. These changes will damage oil quality by increasing acidity, decreasing stability, and degrading the organoleptic characteristics. The fundamental objective during this preservation period is to maintain the fruit without changing the oil characteristics, and significantly increasing the cost of the process.

Leaf removal and washing is necessary to prevent contamination from impurities that affect the organoleptic characteristics of the oil, as well as reducing wear and tear on the mills. The winnowing machine is used as a cleaning system, which separates the impurities of lesser weight than the olive (leaves and twigs) using an adjustable airflow. Water is used for the wash that eliminates heavier objects such as mud, stones, and so on. There are washers that operate by density, adding common salt to the water until the olives start to float, and others that work by trawling, adjusting water circulation rate in such a way that it only carries the fruit.

33.2.2 Paste Preparation

A paste is produced by means of crushing and mixing operations with the objective of separating olive oil as a continuous oily liquid phase from rest of the components of the olive, without changing the composition and organoleptic characteristics.

The crushing breaks down cellular membranes to liberate oil droplets. These droplets group together in variable-sized drops that come into contact with the aqueous

phase present in the paste, which arises from the vegetation water and remains of the washing water. This operation is fundamental for the rest of the extraction process and influences industrial yield and quality of the oil. Today, metallic crushers, generally of the hammer type, have replaced the traditional mills with their cone-shaped millstones.

The olive paste obtained after crushing is mixed slowly (malaxing) with the objectives of gathering together the larger-sized drops of oil, combining into a continuous oily phase, and separating the solid phase and the aqueous phase (Martinez-Moreno and others 1957). To improve yield of this operation, mixers have a heating mechanism that helps to decrease the oil viscosity and makes outflow easier. The temperature in the thermomixer should not exceed 25–30°C to prevent loss of aromatic compounds and an increase in the oxidation process. In order to facilitate the separation of oil from the other components of the fruit (therefore increasing the yield), it is possible to use technological aids in this phase of the process. According to the European rules (Regulation EC 1513/2001) that took effect in November 2003, only one technological aid is allowed, hydrated magnesium silicate, because it has no chemical nor biochemical action, and is therefore commonly the denominated talc (Alba 2004).

33.2.3 Liquid–Solid Separation

This stage constitutes the fundamental part of obtaining oil with the objective of separating liquids contained in the olive paste, that is, the oil and residual water (liquid phase) and the solid mixture (skin, pulp, and broken pits), which is known as "olive-pomace". Three separation systems are used in the industry: selective filtration, extraction by pressure, and extraction by centrifugation.

After mixing, it is possible to obtain an important part of the freed olive oil contained in the olive paste by means of cold extraction (*selective filtration*). The first oil contained in the fruit is thus obtained, which has superior quality characteristics to that obtained later by mechanical extraction. This system is recommended for high-quality oil production but is little used due to its high cost (Hermoso and others 1991).

The *hydraulic press* has traditionally been the system used. It consists, in essence, of placing the paste, once mixed, over some filters made of esparto or synthetic fiber (or a mixture of both), known as pulp mat or olive mat, making up the pressing or load unit. This load, located over a trolley with a central spike, is installed in a container in the press in such a way that, by pressing a load over this container, the filtrate of the liquids contained in the paste is obtained. There are several fundamental steps or factors in the execution of the pressing: paste preparation, its distribution and thickness on the olive mats, the state of the olive mat, specific pressure of the press, and the speed and time of pressing (Martinez-Moreno and others 1964). This system is used in only 3% of Spanish oil mills, whereas in Italy 46% of the industry uses such a method.

The use of centrifugal force is the modern method to carry out the liquid–solid separation. It is carried out in dynamic phase systems where the solids are displaced along the draft shaft and continuously removed. The mixed paste is introduced (partially diluted in water) into a horizontal centrifuge called a decanter. This consists of a conical–cylindrical revolving rotor and a hollow bore helicoidal scraper, which revolves co-axially in its interior and at a different speed. By the action of centrifugal force, the solids are pushed to the interior wall of the rotor and are drawn to the furthest point from the axis of spin, that is to say, the space nearest to the wall of the worm gear motor. Separation continues in this manner until it runs out of liquid. The liquid phases (oil and aqueous)

form concentric rings, which, depending on their density, move further toward the middle. Two outlets, situated at a different level in the periphery of the decanter, are used as exits for the residual water and oil. Therefore, three phases are formed: a solid-olive pomace or *orujo*, and two liquids–oil and residual vegetable water. The system is known as three-phase centrifugation. In Italy it is the system used in 43% of the oil mills, whereas in Spain it only represents 10% (Alba and others 2004).

This system produces a final aqueous phase (residual vegetable water) of approximately 0.7–1.1 L/kg of olives. This effluent contains a high load of contaminants, as assessed by the chemical demand for oxygen, and spillage into public waterways negatively affects flora and fauna (Alba and others 1995). Government measures compel olive mills to put a purification system in place. The first system recommended as an emergency measure was the storage of the residual water in pools for natural evaporation. At the same time, exploitation and purification techniques were developed but were not generally accepted by the sector, due to the level of efficiency and installation and operational costs.

Due to this situation, the consumption of water now tends to be lower to minimize the volume of residual vegetable water produced and thus reducing the environmental problem caused by lack of purification. A variation of the process has been developed called a two-phase centrifugation system, which does not require the fluidity provided by the addition of extra water. The separation of the olive paste is achieved in only two phases, a solid-olive pomace and the other liquid-oil. In this system, the vegetable water of the olive is incorporated into the solid phase, which is distinguished from that obtained by the three-phase process in that it is a more moist and pliable consistency, and is now called two-phase olive-pomace or *alpeorujo* (Hermoso and others 1995). The level of final effluent in this production system is reduced to 0.25 L/kg of olives. In Spain, it is regarded as the system of excellence (87% of cases). However, in Italy it is used only in 11% of the industry.

33.2.4 Liquid–Liquid Separation

The oil obtained in the previous stage contains some amount of residual water and a few solids, in the same way that the residual water contains some oil and a large percentage of solids. It is necessary, therefore, to purify the oil (by freeing solids from the residual water) as well as the residual water to obtain the small amount of oil that it contains. Before separating the liquids, the solids, also known as "fines", are eliminated by using vibratory sieves or filters, to separate the highest possible quantity of "fines". Finally, oil separation from water is carried out by natural decantation, centrifugation, or a combination of both.

The difference in density between the oil (0.915–0.918) and the residual water (1.015–1.086) enables separation by natural decanting into vats or settling pits that are linked together. It is the oldest method, and even today it is still being used in some oil mills. However, it requires a large space and a long period of time, during which the contact of the oil with the aqueous phase can cause fermentation and changes in oil quality.

The use of a vertical centrifuge enables continuous and rapid liquid–liquid separation. By the addition of a specific quantity of water, separation of oil from the rest of the residual water is achieved and, in the same way, but independently, recovery of the oil fraction, which is in the aqueous phase. The factors to be considered in this operation are homogeneity and volume of the liquid to be centrifuged, temperature, volume of added water, and time taken between unloading. The oil that comes out of the centrifuge passes into small decanters for degassing and continues to the recipients, where its quality classification is derived.

33.2.5 Storage

The storage period is limited to a season or part of the following one. Longer periods are only anticipated in industrial mills, which include, as well as the extraction process, bottling and marketing operations. During storage, every possible precaution should be taken to prevent three causes of oil spoilage: contact with unsuitable materials, prolonged contact with aqueous impurities, and oxidation. It is advisable to use vats of a capacity of approximately 50 tons, for easier classification and marketing. Vats must be constructed with impermeable and inert materials so oil will not penetrate or react with the surface, as any absorbed oil that cannot be removed by cleaning will be altered and will compromise further use of the vat. The storage cellar must have minimum luminosity, with walls and floors that are easy to clean and maintained between 15 and 18°C without abrupt changes in temperature. Today, the vats that best accomplish these conditions are called "trujales" or traditional underground vats, which, thanks to an appropriate cover of vitrified refractory tiles, ensure the preservation of the oils. If surface vats are used, they must be covered and protected from atmospheric agents and variations in temperature. The most ideal material is stainless steel, although other materials can be used provided that an inert inner covering is used. Vats must have a conical bottom or a flat incline and a purge tap to facilitate sediment removal.

33.3 INFLUENCE OF PROCESSING ON MAIN CHARACTERISTICS OF VIRGIN OLIVE OIL

Considering the characteristics of the extraction process for virgin olive oil, the variables that can affect the final nature of the product are not very extreme. Among them are temperature and time of malaxing, the system of liquid–solid separation (pressure, two phases, or three phases), or the use of some aids, typically talc. Modifications in some of these parameters during the process originates changes in certain components of the olive that allow tracking of the process. In fact, some of the components of the oil are not present in the fruit, but have been formed during and as a result of the extraction process.

33.3.1 Volatile Compounds

In this group are found all compounds responsible for the characteristic aroma of virgin olive oil, although the volatile compounds can only explain 66% of the sensorial attributes (Morales and others 1999). More than a hundred volatile compounds have been identified, such as hydrocarbons, alcohols, aldehydes, ketones, esters, acids, phenolic compounds, terpene compounds, and furan derivatives. However, there is still no objective method of measuring the aroma, which requires evaluation by a panel of specialist tasters (Panel Test).

In good quality oils, the retained volatile compounds come from the actual fruit and are generated by biogenesis mechanisms. Some compounds are originally found in intact flesh of the olive, while others, called secondary or technological volatiles, are formed during breaking down of the cellular structure as a result of enzyme reactions that take place in the presence of oxygen. The principal biochemistry route is through the action of lipoxygenase, and precursors such as the 18-carbon polyunsaturated linoleic and linolenic fatty acids (Olias and others 1993; Sánchez and Salas 2000). When the harvest conditions of fruit and production or storage of virgin olive oil are defective, a whole series of disagreeable volatile compounds responsible for sensory defects (off-flavors) are generated. Very

different mechanisms are implicated in the genesis of these undesirable compounds. The majority is related to microorganisms (yeast, bacteria, and molds) during storage of fruit before oil extraction, generating volatile compounds responsible for sensory defects such as fusty, musty, or winey-vinegary. During the storage of unfiltered virgin olive oil, sediments are deposited that can also allow fermentation, generating volatile metabolites responsible for the sensory defect known as muddy sediment.

There are also other chemical mechanisms that produce undesirable volatiles. The main one is auto-oxidation of fatty acids. This process is generated spontaneously by the action of atmospheric oxygen, producing a series of chain reactions by a free-radical mechanism. The products of this oxidative change are responsible for rancidity, as well as significant deterioration in nutritional quality.

When the intensity of these sensory defects is high, the oils are classified as "lampante" according to current regulations (EC 2001), require further refining, and are often rejected by the consumer.

The extraction conditions of virgin olive oil have a great influence on its sensory quality. During the centrifugation process, temperature and time of malaxing can be altered to potentially affect quality. Volatile compounds, produced through the lipoxygenase pathway (hexanal, Z-3-hexenal, E-2-hexenal, hexyl acetate, Z-3-hexenyl acetate, hexan-1-ol, E-3-hexen-1-ol, Z-3-hexen-1-ol, and E-2-hexen-1-ol), have been analyzed by dynamic headspace gas chromatography, gas chromatography–mass spectrometry, and gas chromatography–olfactometry (Morales and Aparicio 1999) as a function of different malaxing times and temperatures in an experimental oil mill. The results demonstrate that each volatile responds differently depending on the variables such as time and temperature of malaxing. Nevertheless, the indices proposed in relation to best extraction conditions on the basis of volatile are a temperature of 25°C and a malaxing time of between 30 and 45 min. Higher temperature (T greater than or equal to 35°C) with minimum values of time ($t < 30$ min) could also be useful as an alternative way to obtain pleasant green virgin olive oils.

Nevertheless, it seems that something of controversy exists as far as the conclusions of investigations carried out on the influence of processing on olive oil characteristics. Recently, Tura and others (2004), characterizing the volatile compounds of 40 Australian virgin olive oils, concluded that, except for the effect of malaxing time on the hexanal content, they do not appear significantly different. In this study, an increase in the time of malaxing induces a reduction in the hexanal formation (comparing 15 and 60 min of malaxing). These results agree with results previously reported by Ranalli and others (2001).

The effect of the solid–liquid separation system on the volatile fraction has been studied. Aparicio and Luna (2002) verified that the concentration of almost all the volatile compounds, mostly C_6 and C_5 alcohol, was superior in oils obtained by press than those of three phases. Nevertheless, as commented previously, because of problems of residual water, the three-phases system has evolved to the horizontal centrifuge or decanter of two phases, verifying that this implies a greater content in E-2-hexenal and total aromatic substances (Aparicio and Luna 2002).

33.3.2 Phenolic Compounds

The hydrophilic phenolic compounds constitute a group of secondary metabolites in the plant that present peculiar sensorial as well as beneficial health properties. The principal polyphenols of olive oil are tyrosol and hydroxytyrosol, both derived from hydrolysis of oleuropein.

Also found are a smaller proportion of caffeic, vanillic, siringic, *p*-coumaric, *o*-coumaric, protocatechic, synapic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, and homovanillic acids.

The presence of hydrophilic phenolic compounds in virgin olive oil is strictly related to activities of several endogenous enzymes of the fruit. This explains why its concentration in the oil is strongly affected by the conditions of extraction. In fact, some of the secoiridoid aglycons presents in the virgin olive oil originate during crushing of the fruit (Servilli and others 2004). This is the reason for studying the effect of different variables during the extraction process based on phenolic compounds content in the olive oil.

Thus, it is known that the increase in the time and temperature of processing originates a reduction in the concentration of secoiridoid aglycons (3,4-DHPEA-EDA and 3,4-DHPEA-EA) and phenolic alcohol (Angerosa and others 2001; Di Giovacchino and others 2002). The time of exhibition of pastes to contact with air (TEOPAC) is a parameter that modifies the content of phenolic compounds in the oil (García and others 2001; Servili and others 2003). It seems that when the oxygen levels in the paste are reduced, polyphenoloxidase and peroxidase are inhibited, improving the hydrophilic phenolic compounds in the virgin olive oil (Bianco and others 2002).

In the same way, it has been discovered that oils obtained from pastes with de-stoned olives have superior levels of phenolic compounds if we compare them with the traditional pastes that include stones of the olive in the paste prior to separation of solid–liquid. The hypothesis is that the peroxidase enzyme that degrades hydrophilic phenolic compounds during olive oil processing is highly concentrated in the stones (Montedoro and others 2001).

Aids such as enzymatic preparations during the extraction process also favor the increase in phenolic compounds in the corresponding oils (Vierhuis and others 2001).

Finally, the effect of the selected extraction system on polyphenol content of the corresponding oils has been studied. Years ago, it was reported that oil obtained by pressure, which does not require addition of water to the paste of olives, shows greater levels of polyphenols in comparison with the traditional centrifuged oil (Montedoro and Servili 1992). More recently, the works have been focused in comparing the two-phase and three-phase systems. The three-phase system is much more positive from the point of view of phenols than the decanter producing two phases (García and others 2001; Gimeno and others 2002).

The amount of mass (by time unit) that is introduced into the horizontal centrifuge decanter (DCH), in relation to the theoretical capacity, constitutes a parameter of control and regulation. This is closely related to effectiveness of the DCH, and is of great importance in obtaining a good centrifugal separation, and therefore good yields of elaboration. Maintaining constant the geometric characteristics of the DCH, the variations of the injected volume affect the mass residence time in the interior of the decanter, so that an increase of rate leads to a faster separation using the centrifugal separator. An increase in injection rate probably produces a significant increase in polyphenol content (Jiménez-Márquez and others 1995), because the increase in mass volume implies a reduction of malaxing time of the paste in the thermomixing.

The separated oil in the DCH is not completely clean, but includes suspended mass particles, and water that has not been removed. That elimination is necessary to avoid alterations in the oil, such as an increase in acidity, oxidation of the oil, and so on, taking place during storage. This elimination takes place in vertical plate centrifuges diluting the oil with water to carry out the separation. The more elevated water : oil relation (1.5:1) produces a more intense washing of the oil, resulting in oils with minor polyphenol content. Similarly, in comparing processing at different temperatures (30, 40, and 50°C), a diminution in the polyphenol content occurs when higher temperatures are used (Jiménez-Márquez and others 1995).

33.3.3 Tocopherols

Gimeno and others (2002) have studied the effect that centrifuge systems having: two and three phases have in the content of α-tocopherol. In comparing 60 virgin olive oils of the Arbequina variety, coming from two different harvesting periods and in two different states of maturity, there is definitely no difference in the total content of α-tocopherol. This is in agreement with the earlier works of Ranalli and Angerosa (1996).

Nevertheless, the content of α-tocopherol is affected if it is produced with fruits de-stoned prior to the extraction of virgin olive oil. Recently, Lavelli and Bondesan (2005) have confirmed that using de-stoned fruits (instead of stoned fruits) diminishes the content of α-tocopherols of the corresponding virgin olive oil, which is in agreement with previous works (Frega and others 1997). Although it is important to emphasize the content of tocopherols in virgin olive oil, the effect of the technology is minimal in comparison with genetic factors or degree of maturity.

33.3.4 Chlorophylls and Carotenoids

One could expect that, given their lypophylic nature, all the chlorophylls and carotenoids present in the olive fruit will be transferred to the olive oil. However, it has been demonstrated (Gallardo-Guerrero and others 2002) that the extraction process affects

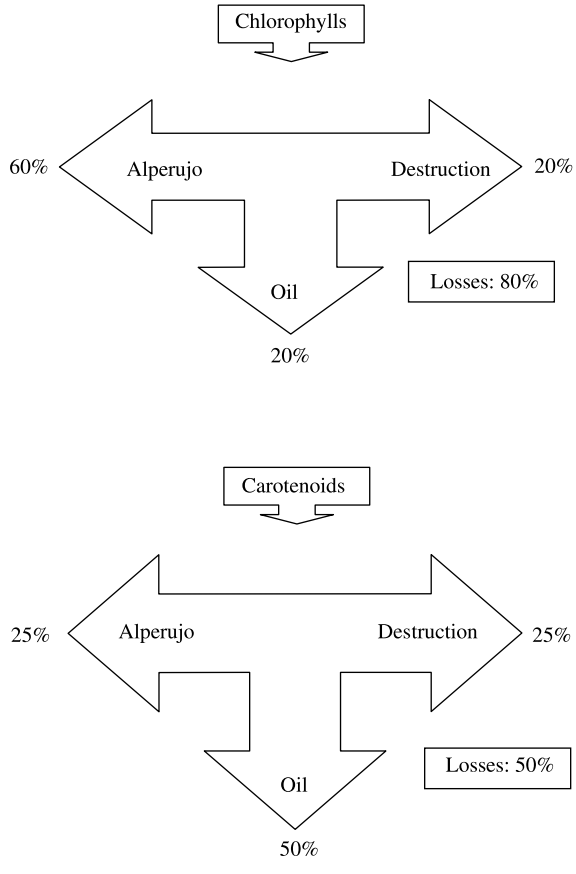

Figure 33.2 *Percentage of transferred, occluded and destroyed pigments during olive oil extraction.*

chlorophylls and carotenoids in different ways (Fig. 33.2). Only 20% of the chlorophylls are transferred to the oil, with 60% of the same fraction occluded in the alperujo and 20% destroyed during the extraction process. On the other hand, 50% of the carotenoids present in the fruit are transferred to the olive oil, 25% of the fraction occluded, and the other 25% destroyed during proccesing.

In general terms, chlorophylls tend to experience chemical and physical modifications as a result of processing and storage of green vegetables. Chlorophyll pigments are particularly affected by acid conditions and heating (Schwartz and others 1981; Mínguez-Mosquera and others 1991; Chen and Chen 1993). Therefore, chlorophylls and their derivatives can be used as treatment and quality markers in processed foods (Gandul-Rojas and others 2000). In the chl (chlorophyll) molecule, the ester of the methoxycarbonyl group in C-13^2 is exceptionally stable towards acids and remains virtually intact during short treatments at room temperatures. At higher temperatures, however, acid conditions lead to hydrolysis of the ester followed by decarboxylation, producing chl pyroderivatives (Hynninen 1991). Although pyrophys are also found in fermented foods such as wild rice or table olives (Schwartz and Lorenzo 1990; Mínguez-Mosquera and Gallardo-Guerrero 1995), the presence of chl pyroderivatives is largely related to the thermal treatment of vegetables, making pyrophys the main pigment in sterilized products (Schwartz and Lorenzo 1991). Teng and Chen (1999) have recently investigated the degradation of chlorophylls and formation of pyrochlorophylls and their derivatives during baking, blanching, steaming, and microwaving of spinach leaves. In olive oil submitted to physical refining by deodorization with nitrogen, pyrophys have also been detected (Gandul-Rojas and others 1999). This fact converts pyrophys into authentic traceability markers of the thermal process and by so much of great interest into the sector of the olive oil to guarantee the smooth thermal conditions in which virgin olive oil should be extracted.

Recently, investigations have been carried out on the effect that the addition of some enzymatic preparations, natural extracts obtained from genetically unmodified organisms, can exert to a greater advantage for the paste and an oil with better qualities as an end result. These preparations are usually a celulolytic, hemicelulolytic, and pectolytic enzyme mixture. Ranalli and others (2005) have studied the effect that some of these preparations have exerted on some chemical compounds in the oil, specifically on the pigments. In fact, the study was carried out with two different varieties of olives, and concluded that the addition of this enzymatic processing aid increases significantly the chlorophyllic and carotenoid fractions in the oils in comparison with control oils.

The increase in malaxing temperature between 20 and 30°C increases the chlorophyll and carotenoid contents. Nevertheless, temperatures greater than 30°C do not imply an increase in the green and yellow chromatic fractions of oils (Ranalli and others 2005). There are only limited investigations into the effect of extraction systems (two or three phases) on oil coloration. Work by Gimeno and others (2002) indicates that the extraction system chosen does not affect the β-carotene content of oils.

33.4 QUALITY MARKERS OF OLIVE OIL

According to the International Olive Oil Council, olive oil is "the oil obtained solely from the fruit of the olive tree (*Olea europaea sativa* Hoffm. Link) to the exclusion of oils

obtained using solvents or by re-esterification processes and of any mixture with oils of another kinds". This oil can be produced commercially under different denominations. Virgin olive oil is "the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions particularly thermal that do not lead to alteration in the oil and which has not undergone any treatment other than washing, decantation, centrifugation and filtration".

To define the quality of olive oil, like any other food product, is a difficult task influenced by a multitude of variables. Generically, it can be defined as the property or set of inherent properties to a thing, that allow to appreciate it like equal, better or worse "than the rest of its type" (Uceda and others 2004).

There are different conceptions of quality according to the use of the olive oil. Established first is that quality is not unique and that it can be different, depending on the point of view: from the regulated viewpoint, which is defined by the established norms, through the nutritional, commercial, sensorial angles.

33.4.1 Regulated

The regulated quality is the easiest to define as it is clearly defined by EC Regulation 2568/91, modified by CE n°. 1989/2003 (6 November), and is shown in Table 33.1. Currently, two rules exist that regulate the denominations of olive oils and olive-pomace oil (EC 2001; IOOC 2001). In both regulations there are parameters, known as quality parameters, to evaluate the quality of the oils and these are free acidity, peroxide index, K_{232}, K_{270}, ΔK, and organoleptic characteristics.

The degree of free acidity measures the percentage of free fatty acids, expressed as oleic acid. It is the index of changes experienced by the fruit during fermentation in the production and storage process.

The peroxide index evaluates the state of primary oil oxidation. The most important causes of a high value of this index are the history of the fruit (soil, frosts) and exposure to factors that cause oxidation (high temperatures, aeration, and the presence of trace metals) during production and storage. It is expressed in miliequivalents of active oxygen by kg of fat.

The K_{232} is a spectrophotometric measure of conjugated dienes, which is used along with the peroxide index to evaluate the primary oxidation of the oil. This parameter only is included in the EC regulation.

TABLE 33.1 Quality Olive Oil Indexes Established by CE n° 1989/2003 (6 November 2003).

	Acidity (%)	Peroxide Index	K_{270}	ΔK	K_{232}	Median Value of Fruity Aroma	Median Value of Defects
Extra virgin olive oil	≤0.8	≤20	≤0.22	≤0.01	≤2.50	>0	0
Virgin olive oil	≤2.0	≤20	≤0.25	≤0.01	≤2.60	>0	≤2.5
Lampante virgin olive oil	>2.0	–	–	–	–	>2.5	–
Refined olive oil	≤0.3	≤5	≤1.10	≤0.16	–	–	–
Olive oil	≤1.0	≤15	≤0.90	≤0.15	–	–	–
Crude olive–pomace oil	–	–	–	–	–	–	–
Refined olive–pomace oil	≤0.3	≤5	≤2.0	≤0.20	–	–	–
Olive–pomaceoil	≤1.0	≤15	≤1.70	0.18	–	–	–

Shading indicates commerical categories.

The K_{270} is used to evaluate, spectrophotometrically, the conjugated trienes and indicate the state of secondary oxidation of the oil. The resulting products of this oxidation (aldehydes and ketones) also absorb at wavelengths of 262, 268, and 274 nm. The ΔK coefficient takes into account these absorbencies and is defined as

$$\Delta K = K_{268} - [(K_{262} + K_{274})/2].$$

Besides physico-chemical parameters, virgin olive oils are also classified by their organoleptic characteristics determined by a group of trained tasters, according to the rules of sensory analysis (Albi and Gutierrez 1991; IOOC 2001). When no defects exist in the flavor (visual together with olfactory–taste–tactile), the tasters evaluate the presence and intensity of positive attributes. The sensory attribute "fruity" is responsible for a mixture of olfactory–taste sensations attributed to fresh and healthy fruit at an optimal degree of ripeness. This attribute is essential in the sensory evaluation because the absence of this attribute excludes the oil from the virgin grade. However, increased intensity of fruity attribute is often accompanied by a perception of high intensities of bitterness and spiciness, which makes the oil unacceptable for direct consumption, requiring mixing with other virgin oils of less intense flavor. On the new sensory evaluation form, proposed in 1996 by IOOC and adopted by the EC in May 2002, the most frequent negative attributes are scored "fusty, musty, muddy sediment and winey-vinegary". Also included in the "Others" section are all other well-defined negative attributes such as "earthy, esparto, pungent, cucumber, grubby, etc". Only fruity, bitter, and pungent are considered positive attributes. Depending on the median value of the defect perceived with greater intensity and the median value of the fruity attribute, the virgin olive oil is classified into distinct categories. A median organoleptic evaluation of the defect (Md) and the fruity attribute (Mf) is made, valuing from 0 to 5:

0 = Total absence;
1 = Almost imperceptible;
2 = Light;
3 = Medium;
4 = Big;
5 = Extreme.

Besides these quality criteria, the Commission regulation (CE) n° 1989/2003 also described purity criteria that include limits for the following parameters: 3,5, estigmastadiene (mg/kg), % *trans* isomers of fatty acids, fatty acid content, ΔECN42, sterols composition and total sterols, erythrodiol plus uvaol (%), waxes (mg/kg), saturated acids in the 2-position of the triglycerides (mg/kg) and sum of translinoleic and translinolenic isomers (%). All the quality and purity criteria should be fulfilled for a specific category of olive oil.

Color is another organoleptic quality characteristic fundamental to virgin olive oil as it is one of the attributes that affects the consumer most at the time of purchase. The first evaluations of color were based on the visual comparison of oil with standard solutions of bromothymol blue (BTB), using an adaptation of methods developed for seed oils (AOCS 1977, 1988; Gutierrez and others 1986). Photometric evaluations at two wavelengths have been proposed for olive oil (Papaseit and others 1986), and also numerical correlation between the chromatic coordinates and the chlorophyll and carotenoid

content (Mínguez-Mosquera and others 1991), as well as with the BTB indices (Moyano and others 1999). Despite the importance of this quality attribute, none of the current regulations includes color.

33.4.2 Chlorophylls and Carotenoids

In recently extracted virgin olive oil, the chlorophylls and carotenoids of the olive must be present, plus those transformed during the oil extraction process. The modifications these pigments experienced during crushing and malaxing of the paste are derived from the release of cytoplasmic components that come into contact with different enzymes and substrates isolated under normal conditions. To a large extent, pigment modification is influenced by the release of acid compounds. For the chlorophyllic fraction, the generalized reaction is the pheophytinization, and for the carotenoids, the isomerization of xanthophylls with epoxides groups like violaxanthin, neoxanthin, and anteraxanthin, which are transformed into their corresponding derivatives 5,8 furanoids, auroxanthin, luteoxanthin, neochrome, and mutatoxanthin, respectively (Mínguez-Mosquera and others 1990). Also, traces of intermediaries of the chlorophyll catabolism, such as 13^2-OH-pheophytin and 15^1-OH-lactone pheophytin are among the oxidation metabolites and chlorophyllides and pheophorbides as intermediaries in the deesterification initiated by chlorophyllase (Gandul-Rojas and Mínguez-Mosquera 1996; Gandul-Rojas and others 2000).

33.5 TRACEABILITY MARKERS OF OLIVE OIL PROCESSING

33.5.1 Phenols and Volatiles

In addition to the studies made on the effect of processing conditions on olive oil exerted on the chemical composition of the same, statistical studies have been made considering as much the fraction of volatiles as the fraction of phenols. Such studies allow discrimination of oils according to extraction method.

In this sense, Angerosa and others (2000) could classify correctly 54 virgin olive oil samples corresponding to three harvesting periods based on the type of extraction: laboratory-scale, industrial two phases, and three phases. By means of principal component analysis (PCA) the variables were selected between volatile and phenol fractions. This allowed the conduction of a linear discriminante analysis (LDA) capable of classifying correctly the samples, based exclusively on the methodology of extraction used.

A similar approach was employed by Servilli and others (2003) to classify (PCA) oils of two Italian varieties obtained with differents time and temperature of malaxing, on the basis of phenolic and volatile composition. Additionally, applying a partial least-squares analysis (PLS) allows the development of a response surface model (RSM), which, besides the explanation of between 89% and 95% of the variance of the two varieties, allows the selection of best conditions (based on volatile and phenolic composition) of malaxing. Thus, for the Frantoio variety a temperature of 22°C and 30 min of TEOPAC consitutes the theoretical optimal conditions, whereas for the Moraiolo variety these conditions are 26°C and 0 min of TEOPAC. Besides confirming that the variety is a fundamental factor in the volatile and phenolic composition of virgin olive oil, the authors

demonstrated the production of a virgin olive oil of high quality. It would be interesting to fit processing parameters to each variety.

33.5.2 Chlorophyll and Carotenoid Indexes

As commented in a previous section, in recently extracted virgin olive oil, only the pigments of the olive must be present, plus those transformed during the process of extraction of the oil. Nevertheless, the small but always important differences found in the carotenoid and chlorophyll metabolism during the ripening of the fruit according to variety, influenced by endogenous enzymatic activities, are going to be reflected in the pigment composition of the corresponding oils. Consequently, the chlorophylls and carotenoids of virgin olive oil become parameters of quality and authenticity for this product. The presence of chlorophylls and carotenoids that are pigments different from those just described, or the detection of a greater degree of transformation is indicative of incorrect or fraudulent practices in processing (Gandul and others 1999).

A study of the chlorophylls and carotenoids in 50 monovarietal virgin olive oils of Spanish origin, besides defining the inherent pigment profile of the virgin olive oil, has allowed the establishment of two parameters of quality that must be fulfilled by these oils (Gandul-Rojas and others 2000). On the one hand, it has been shown that between both pigment fractions exists a constant relation, around the unit, independently of the variety and the state of ripening of the fruit. This relation is maintained within narrow margins, oscillating between 0.5, when the oils come from mature fruits, up to 1.4 when, the oils are obtained from unripe fruits. On the other hand, the carotenoid fraction has a constant relation between lutein, which is the main component of this fraction, and the rest of the minority carotenoids. In all virgin olive oils, the relation of minoritary carotenoids/lutein is located around 0.5 or exceptionally superior to 1 in the oils obtained from olives of the Arbequina variety, and this parameter, in addition to authenticating the quality "virgin olive oils", allows differentiation of varietal origin. In the same way as the relation of chlorophylls/carotenoids in oils obtained from very mature fruits, the relation between minoritary carotenoids and lutein diminish up to values of 0.2 in oils obtained from very mature fruits.

Later studies have allowed the verification of such parameters of quality and authenticity of virgin olive oil, in general, to stay stable after 12 months of storage of the oil at 15°C and in the dark. These are conditions generally used in the industry to keep the oils that are not commercialized immediately after their production (Roca and others 2003).

Although the relation between the fractions of pigments or presence of certain carotenoids stays constant in oils during a year of storage, it has been verified that, during that period, the chlorophyll molecule experiences specific changes that imply modification of the pigments profile associated with the recently extracted virgin olive oil. From the quantitative point of view, losses of pigments do not take place. However, there is a generalized advance of the reactions catalyzed by acids and initiated during oil extraction: the pheophytinization reaction of chlorophylls and the isomerization of the groups 5,6-epoxides of minoriarty xantophylls. Possibly, the greater or smaller content of substances of acid character that are transferred from the fruit to the oil is going to modulate the advance of these reactions (Gallardo-Guerrero and others 2005).

A slight increase of hydroxylation of the C-13^2 of pheophytin *a* takes place, as well as small formation of pyropheophytin *a*, the pigment absent in the freshly extracted oil. It has

been verify that the maximum presence of pyropheophytin in oil stored at 15°C and in the dark for one year is around 3% of the total chlorophyll compounds and that the relation between pheophytin *a* and pyropheophytin *a* must always be superior to 20. In this sense, these small structural pigment transformations, which are not inherent to the extraction process and therefore undesirable, are going to be indications that the oil has been stored. Inadequate conditions of storage such as high temperatures and/or illumination cause increases in the content of these compounds (Gallardo-Guerrero and others 2005).

A combined SPE and HPLC method has been developed, which can be considered as a modification of a previous one (Mínguez-Mosquera and others 1992) in two ways. First, it assures a total recovery of pheophytin *a* and pyropheophytin *a* during SPE sample extraction, and, secondly, it improves selectivity of detection and sensitivity of quantification by using fluorescence detection in tandem with UV–visible spectroscopic detection (Hornero-Méndez and others 2005). This method has allowed the control of "undesired" formation of pyropheophytin in oils obtained with temperatures higher than the recommended ones for virgin olive oil extraction and comparison with the presence or absence in a statistically significant number of oils cataloged as virgin olive oil. Thus, an index of quality of the extra virgin olive oil has been proposed: the calculation of the percentage of pyropheophytin, %Pyrophy = 100*Pyrophy/(Phy + Pyrophy). In the case of reaching values higher than 10%, it would indicate that the oil has been stored at inappropriate temperatures or has been obtained under heat treatments (Gandul-Rojas and others 2005).

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Section X

Fruits and Fruit Juices

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

34

Apple: Production, Chemistry, and Processing

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34.1 Introduction 774
34.2 Supply and Demand 774
34.2.1 Supply 774
34.2.2 Organic Apples 774
34.2.3 Demand 775
34.3 Harvest and Storage 776
34.3.1 Harvest 776
34.3.2 Post Harvest Handling and Storage 776
34.4 Apple Varieties 777
34.5 Physico-Chemical Characteristics 779
34.5.1 Apple Flavor 779
34.5.2 Phenolic Compounds and Antioxidant Capacity 780
34.5.3 Nutritional Quality 782
34.6 Processed Apple Products 783
34.6.1 Apple Juice and Cider 784
34.6.2 Apple Slices 786
34.6.3 Applesauce 787
34.6.4 Processed Frozen Apples 787
34.6.5 Dried Apples 787
References 789

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

34.1 INTRODUCTION

Apple (*Mauls domestica Borkh*) a pome (*Pomoideae* subfamily of *Rosaceae*) fruit is among the top five fruits produced in the world (Table 34.1). Studies support a positive association between fruits and vegetables consumption and good health. Phytochemicals present in fruits and vegetables may protect biological molecules (lipids, proteins, and DNA in our body) from oxidative damage. It is believed that a diet rich in fruits and vegetables can enhance our endogenous antioxidant defenses against chronic degenerative diseases. Apples make a greater contribution than berries to phytochemicals in western diets, because the intake is relatively higher. This chapter describes supply, demand, storage, processing, physico-chemical, antioxidant, and nutritional values of apples.

34.2 SUPPLY AND DEMAND

34.2.1 Supply

Apple is an important commercial tree crop in many countries. The world apple production in 2005 was estimated to be about 63.5 million metric tons. Five countries, China (39.4% of world total), United States (6.8%), Turkey (4.0%), Iran (3.8%), and France (3.3%) accounted for 57% of world apple production in 2005 (Table 34.2). China is a major exporter of fresh eating apples (approximately 0.8 million metric tons in 2004) and apple juice concentrate (0.5 million metric tons) to Europe, North America, Asia, and Africa (Anon 2005).

Apples were the second most valuable fruit crop next to grapes in the United States with estimated farm gate value of $1.78 billion, or 11.5% of all fruit and tree cash receipts of $15.5 billion (USDA 2005). Nearly 100 varieties are grown, however, 15 varieties account for over 90% of the U.S. apple production (Perez and others 2001).

34.2.2 Organic Apples

The EU and the United States together accounted for 95% of the $25 billion in world retail sales of organic food products in 2003 (Dimitri and Oberholtzer 2006). The growth in organic market is due to our rising concern for health and environment. In order for a farm to produce organic apples, the land must be free of any harmful pesticides and fertilizers for a minimum of three years prior to organic production. For a product to be labeled "organic", it must contain over 95% organic materials. A "made with organic" product must contain over 70%.

TABLE 34.1 Leading Fruits and Producing Countries, 2005.

Leading Fruit	World Production (Metric ton, Mt)	Leading Country
1. Banana	72,464,562	India (16,820,000)
2. Grapes	66,533,393	Italy (9,256,814)
3. Apples	63,488,907	China (25,583,934)
4. Oranges	59,858,474	Brazil (17,804,600)
5. Mangoes	27,966,749	India (10,800,000)

Source: FAO (2005).

TABLE 34.2 Apple Production (Mt) in Leading Countries and World.

Country	Average (2000–2005)	2005
1. China	21,583,934	25,583,934
2. United States	4,315,744	4,354,290
3. Turkey	2,383,333	2,550,000
4. Iran	2,338,169	2,400,000
5. France	2,175,184	2,213,000
6. Italy	2,061,605	2,194,875
7. Poland	2,195,917	2,050,000
8. Russia	1,865,333	2,050,600
9. Germany	1,859,483	1,600,000
10. India	1,308,333	1,470,000
World (total)	59,690,201	63,488,907

Source: FAO (2006).

In the United States, organic apple acreage increased from 12,770 acres in 2000 to 17,272 acres in 2001. Washington (6540 acres) California (4529 acres), Arizona (2800 acres) and Colorado (1535 acres) were leading U.S. states in organic apple production. Outside the United States, Europe (8675 acres in 2000), New Zealand (2873 acres), South America (1385 acres), Canada (800 acres) and Turkey are expanding organic apple acreage. Two other factors, premium price to growers for organic apples and organic production as a way to prepare for the loss of production tools such as pesticides are cited for the growth of organic farming (Granatstein and Kirby 2002).

34.2.3 Demand

Apple products from across the globe are available in the United States. About 37% of apples produced in the United States are utilized as fresh (Table 34.3). The remaining 63% is processed as, juice and cider (49%, or 75% of all processed), canned apples (mostly applesauce, 10%), frozen and dried (2% each), and jam, jelly, apple butter, etc. (about 1%). Next to oranges (83.5 lb), apples (50.4 lb) were the second most utilized fruit in the United States during 2004 (Table 34.4).

TABLE 34.3 Per Capita Consumption of Apple and Apple Products in United States[a].

	Fresh	Canned	Juice/Cider	Frozen	Dried	Others	All
2000	17.5	4.4	21.4	0.7	0.8	0.3	45.0
2001	15.6	4.6	21.3	0.9	0.8	0.2	43.4
2002	16.0	4.0	21.4	0.7	0.8	0.2	43.1
2003	16.9	4.5	23.1	1.0	0.6	0.4	46.5
2004	18.6	4.5	25.4	0.9	0.7	0.3	50.4
Average	16.9	4.4	22.5	0.8	0.7	0.3	45.7
% Share	37.00	10.0	49.0	1.8	1.5	0.7	

[a]lb, Fresh weight equivalent.

Source: USDA (2005).

TABLE 34.4 Per Capita Consumption of Fresh and Processed Fruits in the United States[a].

Fruit	2000	2001	2002	2003	2004
1. Orange	90.5	93.1	77.5	83.0	83.5
2. Apple	45.0	43.4	43.1	46.5	50.4
3. Grapes	49.9	45.0	51.2	47.5	48.8
4. Pineapples	12.6	12.2	13.1	14.2	12.7
5. Grapefruit	13.2	13.2	11.6	10.1	10.0
6. Peach	9.8	9.3	9.6	9.1	9.2
7. Strawberries	6.2	9.3	6.0	6.9	6.9
8. Pear	6.2	6.2	5.7	5.7	5.5

[a]lb, fresh weight equivalent.

Source: USDA (2005).

34.3 HARVEST AND STORAGE

34.3.1 Harvest

Apples are harvested at maturity. Farmers use "days after full bloom (DAFB)" to predict harvest maturity. Gala and Fuji apples have "DAFB" of 110–120 days and 170–185 days, respectively. In the United States early season apples are ready for harvest in August. However, peak apple harvest is from September to October. The date of first harvest in New Zealand is February to March (McGhie and others 2005). A low-chilling requirement apple "Anna" in Israel is harvested in June (Lurie and others 2002). In India where apples are grown only in the three mountainous states (Jammu & Kashmir, Himachal Pradesh, and Uttranchal Pradesh) harvesting can be as early as June, or as late as November (Deodhar and others 2006).

Among objective tests for harvest maturity, firmness of apple flesh measured by a pressure tester is considered reliable. Apples for storage and processing typically have pressures above 15 lb, while a pressure in the 13 lb range is suitable for fresh use fruits (Anon 2004). Sensory and other objective measurements such as skin color and appearance, fruit size, soluble solids (Brix) and acidity (% malic acid), starch and internal ethylene concentration (especially for apples to be stored in CA storage) are also taken into account to determine harvest maturity.

Premature harvesting of apples affects flavor, color, size, and storability (susceptibility to bitter pit: dark, dry spots, or pits near or below the fruit surface; and storage scald: brown patches on the fruit surface). Late harvesting causes softer fruits and a shorter storage life.

Preharvest foliar spray of calcium is shown to maintain fruit firmness and decrease physiological disorders such as water core, bitter pit, and internal breakdown. Application of bioregulators such as ethephon may also improve fruit quality and storability (Drake and others 2002).

Apples are picked by hand to minimize bruising. Fresh market apple should be free of bruises, blemishes, and other defects. Bruising during harvest, transport, grading, storage, and packing affect returns to growers, and bruised fruits are generally used for processing into juice and other products.

34.3.2 Post Harvest Handling and Storage

Post harvest treatments of apples involve, washing, sorting, grading, waxing, packing, and cooling. Apples are stored at 0° to -1°C with 92–95% humidity in regular cold storage for a short period. Prompt cooling prevents softness in apples. Under controlled atmosphere (CA) storage, apples can be stored for one year without any appreciable loss of quality.

The CA storage requires airtight refrigerated rooms that are sealed after apples are stocked up inside (and must not be opened for at least 90 days after the seal is affixed). In CA storage, oxygen (O_2) is reduced from atmospheric 21% to 1–3%, and carbon dioxide (CO_2) is increased from atmospheric 0.25% to 1–3%. Relative humidity in the CA storage is maintained at 92–95%.

Storage disorders (loss of firmness, browning, bitter pit, superficial scald, water core, chilling injury, etc.) are not uncommon and result from inconsistent storage protocols and questionable fruit quality going into long-term storage (Anon 2004).

As expected, apples from normal cold (NC) storage are softer than those stored in CA storage (Lopez and others 2000). Lavilla and others (1999) reported improvement in sensory qualities of apples when the CA atmosphere had a low (1.8–2% O_2/1.8–2% CO_2) and ultra-low (0.8–1% O_2/0.8–1% CO_2) as compared to standard oxygen (2.8–3.0% O_2/2.8–3% CO_2).

Use of coatings (carnauba-shellac mixture and candelilla having intermediate gas resistance; shellac and shellac-protein with highest gas resistance) can extend shelf life of apples (Saftner 1999). Coatings can create elevated CO_2 and reduced internal O_2 concentrations similar to CA storage and modified atmosphere (MA) packaging. However, use of coatings can also affect flavor by regulating their synthesis and minimizing loss by evaporation from the fruit surfaces. In Braeburn and Granny Smith apples, gas resistance coatings produced anaerobic fermentation and ethanol. A wax coating with intermediate gas resistance gave increased fruit-like and apple-like volatiles (Bai and others 2002).

As a post harvest treatment, Golden Delicious, Ida Red and McIntosh apples were dipped in sucrose polyester solutions and stored at 5°C with 90–95% RH for four months (Chai and others 1991). Ripening was delayed as evidenced from little loss of tissue firmness and color in Golden Delicious and McIntosh apples.

Use of ethylene action inhibitors such as 1-methylcyclopropane (MCP) has been investigated to study changes in apple quality. A delay in softening, retention of green color and reduction in acid degradation were observed in MCP treated apples (Defilippi and others 2004).

34.4 APPLE VARIETIES

Size, shape, color, flavor, crisp-juicy texture, post harvest storability, etc., are important varietal characteristics. Varieties are grouped as, fresh market, juice and cider, baking, processing, and all-purpose apples. During planting trials apples are screened for, disease resistance such as, susceptibility to bitter-pit, soft scald, sunburn, bruising, fire blight, etc., fruit quality (firmness, size, appearance, juiciness, sweetness, tartness, aroma, and flavor), yield, and storability. Growers receive higher returns for apples going into the fresh market than processing (Table 34.5). There are several all-purpose apples suitable for fresh consumption and processing (Table 34.6).

TABLE 34.5 Season-Average Grower Price (cent/lb) of Apples in the United States.

	2000	2001	2002	2003	2004
1. Fresh	20.0	18.0	24.0	26.0	28.0
2. Canned	7.4	7.0	8.0	7.7	7.4
3. Juice and cider	3.7	4.2	5.2	5.2	4.0
4. Frozen	7.5	7.0	8.8	8.7	8.6
5. Dried	3.6	4.2	5.4	5.4	3.9

Source: USDA (2005).

TABLE 34.6 Characteristics of Selected Commercial Apple Varieties.

Variety	Characteristics	Use
1. Braeburn	Developed in New Zealand in 1952; harvest – September to October; color – typical greenish-gold with red sections; sweet-tart, spicy flavor; yellow flesh; texture is crisp and juicy.	For fresh eating and processing as sauce, pies, baking and freezing, juice, and cider.
2. Empire	A cross between McIntosh and Red Delicious; harvest – late September to early October; dark red appearance; mild tart; crisp; flesh is creamy white.	A good apple for fresh eating and for salad, can be used in other applications as well.
3. Fuji	Developed in Japan in 1962; parentage- Ralls Janet and Red Delicious; harvest – September to late October (in some regions late October to mid-November); appearance – golden hued to red; round shape; sweet and aromatic; crisp texture; flesh is whitish yellow; a good storage apple.	A good apple for fresh eating, salad, sauce, pies, baking, and freezing.
4. Gala	Developed in New Zealand in 1965; harvest – mid to late August; appearance – creamy yellow with red/orange stripes; mild sweet flavor; flesh is yellow, firm, and juicy; a right size apple for snacking.	For fresh eating and salads, it is also a good apple for applesauce and baking.
5. Golden Delicious	Introduced in 1914; Origin, West Virginia, United States; a chance seedling, believed to be due to Golden Reinette and Grimes Golden; harvest – mid-September to early October; pale greenish-yellow appearance; sweet apple with excellent flavor; juicy yellow flesh.	An all-purpose apple; skin is soft and thin for fresh eating; used in salads, applesauce, baking, and fresh apple cider.
6. Granny Smith	Credited to an Australian grand mother Maria Ann Smith; a long season fruit, may not ripen before frost, harvest – October to early November; appearance – signature green; size – large; a slightly tart apple with a crisp texture and white flesh.	Has good eating and cooking qualities, excellent for applesauce, salad, and apple juice.
7. Ida Red	Developed from Jonathan and Wagener; harvest – mid to late October; large apple; appearance, bright red; tart flavor, flesh is white, firm and juicy.	Used as fresh, frozen, canned, sauces, and pies.
8. Jonagold	A "Jonathan × Golden Delicious" cross; harvest – mid October; size – medium large; appearance is orange red; well-flavored greenish white flesh; good taste; crisp and juicy.	Excellent fresh eating and cooking (pie and baked) apple.
9. Jonathan	Discovered as a chance seedling in 1820s on a farm in Woodstock, New York; named after the man who promoted it; harvest – mid-September to mid-October; crimson color with touches of green; flavorful with small to medium slices; flesh is off-white, sweet and juicy; blends well with other varieties in sauces and cider; stays firm during cooking.	All purpose apple suitable both for fresh eating and for applesauce, and pie.
10. McIntosh	An important commercial variety; harvest – early to mid-September; appearance red, striped; white juicy flesh, tender skin, medium large fruit; good aroma.	Mainstay of fresh cider; for eating out of hand and sauce making.

(*Continued*)

TABLE 34.6 *Continued.*

Variety	Characteristics	Use
11. Mutsu (Crispin)	Origin, Japan; a cross between Golden delicious and Japanese variety Indo; one of the later variety apple with mid-October harvest; Light green to yellow; large fruit; moderately sweet; creamy flesh.	Preferred use: fresh, pies, and baked.
12. Northern Spy	Harvest – mid October; large size; yellow–green skin and creamy white flesh; crisp and juicy; spicy, aromatic flavor.	Can be used in various processing, including, slices, sauces, and pie.
13. Pink Lady	Origin, Australia; introduced in 1985; a cross between Golden Delicious and Lady Williams; harvested – late October; appearance bright pink and thus the name; firm, crisp flesh and sweet-tart flavor.	Excellent apple for fresh, salad and sauce use; good for baking and freezing.
14. Red Delicious	Believed to be the most popular variety in the world and in the United States; discovered as a chance seedling on a farm (of a non-apple region) in central Iowa; glossy red with a distinctive "typey" five-pointed elongated shape. Sweet and flavorful; harvested late August; can generally be found in market in Fall and early winter; good aroma apple.	For fresh eating and salads.
15. Rome	Origin, Rome township, Ohio; harvest – mid to late October; size – large; bright red skin; sweet, juicy, white flesh; low acidity.	Good processing apple for pies, sauce, and as baked.

Sources: www.michiganapple.com; www.applejournal.com; www.paapples.org/varieties.htm; www.raa.nsw.gov.au; www.bestapples.com/varieties.

34.5 PHYSICO-CHEMICAL CHARACTERISTICS

Apples contain about: 85% water, 14% carbohydrate, 2.4% dietary fiber, 0.3% protein, 0.20% lipids, and 0.0% cholesterol (Table 34.7). Some variation in these components is expected due to variety, maturity, growing location, agronomical, and environmental conditions.

Approximately 75% of carbohydrates in apples are sugars, fructose (about 6%), glucose (2.4%), and sucrose (2.0%). Sugar alcohol, sorbitol (0.2%) is also present (Gorsel and others 1992). Malic acid is the predominant organic acid (0.3–1.0%). Variety, maturity, and environmental conditions during growth and storage influence acidity of apples (Ackermann and others 1992).

Table 34.8 shows physico-chemical qualities of apple slices stored for 12 days at 2°C. Firmness of Empire apples held better (only 8% loss) than Golden Delicious and Rome (both lost 32% of their original firmness). The flesh colors of Empire and Golden Delicious were better than Rome.

34.5.1 Apple Flavor

Various esters, alcohols, aldehydes, ketones, and terpenoids formed due to biochemical and metabolic reactions during growth, ripening and storage impart characteristic flavor and aroma to fruits. A majority of flavor compounds in apples are volatile esters (Lopez and others 2000). Compounds such as ethyl propionate and butyl acetate give

TABLE 34.7 Approximate Composition of Apples (Raw, with Peel).

Component	100 g	154 g/one Serving
1. Water	85.56 g	131.76 g
2. Protein	0.26 g	0.40 g
3. Total lipids	0.17 g	0.26 g
5. Ash	0.19 g	0.29 g
4. Total Carbohydrate[a]	13.81 g	21.27 g
5. Sugars (total)	10.39 g	16.00 g
6. Sucrose	2.07 g	3.19 g
7. Glucose	2.43 g	3.74 g
8. Fructose	5.90 g	9.09 g
9. Starch	0.05 g	0.08 g
10. Dietary fiber	2.40 g	3.70 g
11. Calcium	6.00 mg	9.00 mg
12. Iron	0.12 mg	0.18 mg
13. Magnesium	5.0 mg	8.00 mg
14. Phosphorus	11.0 mg	17.00 mg
15. Potassium	107 mg	165 mg
16. Sodium	1.0 mg	2.0 mg
17. Zinc	0.04 mg	0.06 mg
18. Vitamin C	4.60 mg	7.10 mg
19. Vitamin A	54.0 IU	83.0 IU
20. Cholesterol	0.0 mg	0.00 mg
21. Calorie	52.0 Kcal	80.0 Kcal

[a]By difference.
Source: USDA National Nutrient Database (2003) (http://www.nal.usda.gov/fnic/foodcomp/).

the characteristics "apple" flavor. Hexyl acetate, imparts "sweet-fruity" note, and 1-butanol, a sweet sensation. Cunningham and others (1986) showed β-damascenone (flower like aroma), butyl isomyl and hexyl hexanoates, along with ethyl, propyl, and hexyl butanoates, important to apple flavor. Other apple flavor compounds are, propyl acetate, butyl butyrate, t-butyl propionate, 2-methylpropyl acetate, butyl acetate, ethyl butyrate, ethyl 3-methylbutyrate, and hexyl butyrate.

The apple flavors are maintained during CA storage (Lavilla and others 1999; Table 34.9).

Compounds responsible for undesirable flavors such as acetaldehyde (piquancy), trans-2-hexanal and butyl propionate (bitter), 3-methylbutylbutyrate and butyl 3-methylbutarate (rotten) were not found in apples analyzed by Lopez and others (1998).

34.5.2 Phenolic Compounds and Antioxidant Capacity

Apples are rich in phenolics compounds (Sun and others 2002), which are secondary plant metabolites having antioxidant properties. Apples differ in their concentration of phenolic compounds (Sluis and Others 2001). The popular Red Delicious apples contain about 240 mg total phenolics per apple (Table 34.10; McGhie and others 2005). Flavonols are present more in peels than in flesh, while hydroxycinnamics such as chlorogenic acid is present more in flesh than in peel (Tsao and Others 2003; Table 34.11). Wolfe and others (2003) reported apple peels to more effectively inhibit the growth of $HepG_2$ human liver cancer cells than other apple components.

Brands of apples juice differ in their phenolic content, and fresh apples juice has more phenolics than stored juice (Table 34.12).

TABLE 34.8 Physico-chemical Characteristics of Apple Slices from Selected Varieties Stored at 2°C.

Storage Day	Empire	Golden Delicious	Rome
	Respiration Rate (mL CO_2/kg · h)		
0	5.56	5.08	6.56
3	1.76	2.14	1.76
7	1.78	1.97	1.78
12	2.12	2.88	2.12
	Firmness (Newton)		
0	2430	1823	2660
3	2353	1783	2360
7	2300	1503	2376
12	1983	1246	1813
	Color (Lightness, L Value)		
0	72.9	73.9	70.7
3	66.0	68.8	58.6
7	66.0	68.4	59.0
12	66.2	68.5	58.8
	Soluble Solids (Brix)		
0	13.9	15.2	14.1
12	13.8	15.0	14.0
	Titrable Acidity (%, as Malic)		
0	0.48	0.32	0.38
12	0.45	0.30	0.35

Source: Kim and others (1993).

TABLE 34.9 Flavor Profile of Granny Smith Apples[a].

Compound	Concentration (μg/kg)
1. Methyl acetate	178.5 ± 78.5
2. Hexyl acetate	70.5 ± 18.9
3. 2-Methylbutyl acetate	81 ± 2.8
4. Ethyl propionate	120.0 ± 69.3
5. Ethyl butyrate	147.5 ± 48.8
6. Hexyl butyrate	25.5 ± 0.7
7. Ethyl 2-methyl butyrate	167.0 ± 19.8
8. Hexyl 3-methyl butyrate	35.0 ± 5.7
9. Ethyl hexanoate	169.0 ± 67.9
10. 1-Propanol	56.5 ± 7.8

[a]After 1 day at 20°C followed by three months under controlled atmosphere (2% O_2/2% CO_2) storage.

Source: Lavilla and others (1999).

TABLE 34.10 Concentration (mg/apple) of Phenolics in Selected Apples[a].

Polyphenolics	Pacific Queen	Red Delicious	R. Gala	Granny Smith
1. Total flavanols	40.7	43.5	24.3	36.9
2. Total flavonols	33.0	26.1	26.0	29.6
3. Phloridzin	2.6	9.3	1.7	1.5
4. Total procyanidins	100.8	123.3	60.1	45.6
5. Chlorogenic acid	80.3	34.5	27.6	27.6
6. Anthocyanin	5.7	6.5	2.8	0.1
7. Total phenolics	263.1	243.2	142.5	141.4

[a]Calculated for both flesh and skin.
Source: McGhie and others (2005).

TABLE 34.11 Average Concentration (μg/g Fresh Weight) of Phenolic Compounds in Peel and Flesh of Apple[a].

Compound	Peel	Flesh
1. Total hydroxycinnamics[b]	148.5	193.0
2. Total procyanidins[c]	958.2	267.7
3. Total flavonols[d]	288.2	1.3
4. Total dihydrochalcones[e]	123.7	19.3
5. Total polyphenolics (HPLC)[f]	1604.4	481.3
6. Total phenolic content (F-C)[g]	1323.6	429.6

[a]Based on eight apple varieties; [b]Chlorogenic and p-coumaroylquinic acid; [c]Catechin, epicatechin and other procyanidins; [d]Quercetin-3-galactoside, glucoside, xyloside, arabinocide, rhamnoside; [e]Phloridzin and ploreitin; [f]Measured by HPLC; [g]Measured by F-C method.
Source: Tsao and others (2003).

TABLE 34.12 Concentration of Phenolic Acids, Flavonoids and Total Polyphenols (mg/L) in Fresh and Stored Apple Juice.

Component	Fresh Juice	Stored Juice
1. Phenolic acids	43.5–93.1	34.4–84.5
2. Flavonoids[a]	20.3–92.1	17.6–74.8
3. Total polyphenols	63.8–163.4	52.0–139.4

[a]Quercetin glucosides, phloridin, and kaempferol.
Source: Gliszczynska–Swiglo and Tyrakowska (2003).

Total antioxidant capacity (TAC) of selected apples was reported as, 3578–5900 μmole of Trolox Equivalent (TE)/serving of an apple (Wu and others 2004; Table 34.13). As expected, apples with peel had higher TAC values than without peel. Red Delicious apples had more TAC (5900 μmole) than Granny Smith (5381 μmole), Gala (3903 μmole), Golden Delicious (3685 μmole) and Fuji (3578 μmole).

34.5.3 Nutritional Quality

Apples are a natural source of sugars and dietary fiber (80% of which are soluble fibers) various minerals and vitamins (Table 34.7). Fresh eating apples have little fat and cholesterol, and contribute less than 100 calorie per serving size. Table 34.14 shows nutrient profile of various apple products.

TABLE 34.13 Total Antioxidant Capacity and Total Phenolics in Selected Apples.

Apple	%M[a]	TAC[b]	TP[c]	Serving Size One Fruit (g)	TAC/s[d]
1. Fuji	84.2	25.93	2.11	138	3,578
2. Gala	85.8	28.28	2.62	138	3,909
3. Golden Delicious (with peel)	86.1	26.70	2.48	138	3,685
4. Golden Delicious (without peel)	86.9	22.10	2.17	128	2,829
5. Red Delicious (with peel)	85.5	42.75	3.47	138	5,900
6. Red Delicious (without peel)	86.7	29.36	2.32	128	3,578
7. Granny Smith	85.7	38.99	3.41	138	5,381

[a]% Moisture; [b] Total (lipophilic and hydrophilic antioxidant capacity as Trolox equivalent (TE)/g; [c] Total phenolics as mg gallic acid equivalent/g; [d] Total antioxidant/serving size.

Source: Wu and others (2004).

TABLE 34.14 Nutrient Profile of Apple Products.

Nutrients/100 g	Apple Juice[a]	Apple Juice Concentrate[b]	Apple Canned[c]	Apple Sauce[d]	Dehydrated Apple[e]	Infused Dried Apple[f]
Calories (kcal)	47.0	166.0	67	43	346.0	336.0
Total fat (g)	0.11	0.37	0.43	0.05	0.58	1.93
Saturated fat (g)	0.019	0.06	0.07	0.008	0.095	0.20
Polyunsaturated fat (g)	0.033	0.108	0.126	0.014	0.171	0.30
Monounsaturated fat (g)	0.005	0.015	0.017	0.002	0.024	1.4
Cholesterol (mg)	0.00	0.0	0.0	0.0	0.0	0.0
Sodium (mg)	3.0	25	3.0	2.0	124.0	31.0
Potassium (mg)	119.0	448	70.0	75.0	640.0	64.0
Total carbohydrate (g)	11.68	41.0	16.84	11.29	93.53	81.7
Total fiber (g)	0.1	0.4	2.0	1.2	12.4	6.3
Total sugar (g)	10.90	38.83	14.84	10.09	81.13	75.5
Sucrose (g)	1.70	NA	NA	NA	NA	NA
Glucose (g)	2.50	NA	NA	NA	NA	NA
Fructose (g)	5.60	NA	NA	NA	NA	NA
Protein (g)	0.06	0.51	0.18	0.17	1.32	2.26
Calcium (mg)	7.0	20.0	4.0	3.0	19.0	65.0
Iron (mg)	0.37	0.91	0.24	0.12	2.00	0.42
Vitamin C (mg)	0.9	2.1	0.2	1.2	2.2	372.0
Vitamin A (IU)	1.0	0.0	56.0	29.0	81.0	20.0
Water (g)	87.93	57.0	82.28	88.35	3.0	12.7

[a]Canned or bottled, unsweetened, without added ascorbic acid; NA: Not Available. [b]Frozen concentrate without added ascorbic acid; [c]Canned, sweetened sliced apple: drained and heated; [d]Canned unsweetened apple sauce without added ascorbic acid; [e]Low-moisture dehydrated, sulfured apples; [f]Courtesy of Graceland Fruit Inc, Frankfort, MI, United States.

Source of other data: http://www.nal.usda.gov/fnic/foodcomposition/cgi-bin/list_nut_edit.pl.

34.6 PROCESSED APPLE PRODUCTS

Processed apples such as apple juice, cider, applesauce, jam, and jelly are well-known products. Frozen diced and sliced apples are used in pies and other baked applications. Minimally processed apple slices are retailed as snack items. Intermediate moisture and infused dried apples are used as ingredients in bakery, dairy products, cereals, snack

bars, confection, etc. Infused processed frozen and stabilized apples are used in dairy and bakery products. This section describes processing of selected apple products.

34.6.1 Apple Juice and Cider

In the United States apple juice is the most dominant of all processed apple products (Perez and others 2001). Apple juice-making has traditionally been a mechanical process involving milling/mashing of apples into smaller pieces and pressing with a hydraulic press to express juice. Thus, apple juice and cider are extracted liquids containing soluble solids primarily fruit sugars and acids. Cider is cloudy and amber brown because suspended solids are not removed by filtration. Unpasteurized ciders are a great safety concern. Unlike a "hard cider," which contains a little alcohol, regular ciders are not fermented. The characteristic cider flavor varies from region to region and is based on types of apples used in the manufacturing process.

Apples used in juice-making are generally culled from fresh packing lines. In traditional milling-pressing operations, the yields are 70–80% (w/w), and even lower (~65%) for stored apples (Cliff and others 1991). However, through use of enzyme liquefaction and membrane filtration, the apple juice yields can be as high as 98%.

Process for apple juice would vary among processors. However, basic juice-making involves (Fig. 34.1): Fruit receiving: air cleaning, washing, inspection-grading (to remove rotten and moldy fruits), quality checks (weigh, Brix, acidity, etc.) → milling/slicing (to crush/mash/ using bars/knives) → pressing/extraction → enzyme treatments or use of fining steps, or both (to remove suspended solids) → clarification/membrane filtration → pasteurization → packaging (hermetically sealed in cans or bottles), or asceptically processing and packaging.

Usually a blend of several apples is used to provide flavorful apple juice and cider. A batch hydraulic press (a traditional rack and frame press where milled apples are placed in thick fabrics separated by wooden racks and hydraulic pressure is applied from the top of the rack to release the juice) and various types of continuous pressing equipments can be used. The latter are often employed in commercial operations because of efficiencies in yield, quality, and process controls. It also allows low residual moisture in the pressed cake or pomace.

Commercial juice press designs include a belt press where milled apple pulp is held between belts and juice is released through pressure from rollers, and a screw press made up of tapered screws to convey and squeeze juice against close fitting screens. Juice extraction can also be done using a centrifuge. In a counter current extraction process, juice is extracted using liquids such as water to remove juice solids. This extracted water is then depectinized and passed through membrane separation systems to generate about 10–14 Brix juice, which can be further concentrated.

Presence of colloidal pectic substances such as starch, cellulose, hemicelluloses, pectins, etc., cause haze, cloudiness, and sedimentation in apple juice. Therefore, after extraction, apple juice is treated with enzymes, fining agents, or both, to remove suspended solids and clarified using membrane filtration.

Two pectinase enzymes are usually employed in juice making operations. (1) Polygalacturonase (endo-form: EC 3.2.1.15; and two exo-forms: EC 3.2.1.67 and EC 3.2.1.82), which act on glycosidic linkage and are responsible for fruit ripening. These enzymes can be used as a press aid. (2) Pectin lyase (EC 4.2.2.10) it degrades the pectin chain by acting on glycosidic linkage through β-elimination. A combination of endo- and exo-lyases can be used to degrade pectin as a clarification and membrane

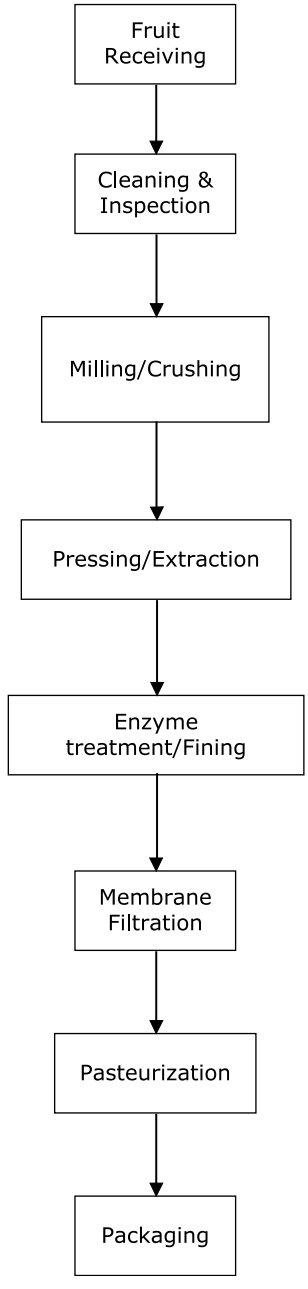

Figure 34.1 *Process for apple juice.*

filtration aid. The enzyme doses, time, and temperature would depend upon specific enzymes; the clarification time is inversely proportional to the enzyme concentration used.

Membrane filtration such as ultrafiltration capable of retaining particles of about 0.005 micron (molecular weight >1000 Dalton) or larger (components such as plant gum, proteins, enzymes, gelatin, etc., operating pressure of 70–200 PSI) but allowing low

molecular weight particles such as sugars, salts, and acids to pass through has become the industry standard to remove haze-forming particles following an enzyme treatment or use of various adsorbents or fining agents (diatomaceous earth, gelatin, etc.).

Presence of amino acid proline has been linked with haze formation. A protein-polyphenol haze is due to interactions between a protein fraction capable of binding polyphenols (haze active protein) and a polyphenol fraction (haze active polyphenol) that can form a bridge between two protein molecules. Stabilization steps for manufacturing a clear apple juice are designed to reduce the levels of either protein or polyphenol or both (Siebert and Lynn 1997, 2000).

Following the enzymic treatment and membrane separation the juice is pasteurized ($\sim 88^{\circ}C/1$ min) and packaged. A large part of apple juice is sold as frozen concentrate as well.

In 2001, the FDA promulgated rules requiring juice processors to use HACCP principles and sanitary standard operating procedures (SSOPs) because of the risk of food-borne illness from consumption of unpasteurized juice products. Under the federal Juice HACCP rule, juice processors must comply with: (1) HACCP principles and systems; and (2) reduce a theoretical population of "pertinent" microorganisms in the juice by five-log cycles. The "pertinent" microorganism is defined as the most resistant microorganism of public health concern that is likely to occur in juice. *E. coli* 0157:H7 and *Cryptosporidium parvum* are regarded as "pertinent" organisms for apple juice.

In 1996, *E. coli* 0157:H7 contamination of apple juice was responsible for over 70 illnesses and one death in the northwest. This organism due to its acid tolerance and low infectious dose (10–2000 cfu/g) is of concern in unpasteurized juices and ciders. Chikthimmah and others (2003) reported a five-log cycle reduction in *E. coli* 0157:H7 in cider by adding fumaric acid (0.15% w/v) and sodium benzoate (0.05% w/v) to the product. The use of these preservatives lowered the natural pH of cider (i.e., 3.40–3.87) to pH 3.19–3.41 and inactivated any *E. coli* organisms in the product.

Patulin a mycotoxin (one of the microbial hazards to be controlled under HAACP regulation) produced by certain species of *Penicillium*, *Aspergillus*, and *Byssochylamys* molds that may grow on harvested apple is a regulatory concern. The regulations typically limit patulin content in apple juice to no more than 50 μg/kg (Kryger 2001).

34.6.2 Apple Slices

A number of different techniques are used to maintain flesh color, flavor, and crisp texture of apple slices. The softening of apple tissue due to loss of cell fluids and enzymatic browning are subject of many investigations including modified atmosphere packaging, low temperature storage, and addition of preservatives (Soliva-Fortuny and others 2001).

Polyphenol oxidase (PPO) catalyzed oxidation of phenolic compounds present (enzymatic browning) in apples into o-quinines, which polymerize to produce dark brown color when apple slices are exposed to air. The role of oxygen in enzymatic browning and cellular damage was evident from lower firmness values and greater amount of cell fluid's loss in apples packed in a 2.5% O_2 and 7% CO_2 atmosphere package than in 100% N_2 atmosphere package (Soliva-Fortuny and others 2003). Commonly used effective inhibitors of PPO are sodium metabisulfite and ascorbic acid. However, Sulfites have been linked to allergic reaction, especially in asthmatic individuals. The FDA regulates the use of sulfites in foods and sulfite content ≥ 10 ppm is required to be shown on the labels. The alternatives to sulfites are based on ascorbic acid, citric acid and calcium salts. Santerre and others (1988) reported ascorbic acid or D-araboascorbic acid/citric acid and calcium chloride combinations effective in preserving color of frozen apple slices.

Calcium ascorbate or calcium erythorbate and ascorbic acid applications and storage at −7–20°C have been claimed to extend shelf life of fresh cut fruits including apples (Chen and others 1999; U.S. patent 5,939,117). Powrie and Hui 1999 (U.S. patent 5,922,382) described a method of preserving fresh apple by immersing apple pieces in an acid solution containing 5–15% ascorbic acid and erythorbic acid (pH 2.2–2.7) for up to 3 min, followed by removal of excess solution from fruit surfaces, quick chilling and storing at 0–10°C.

34.6.3 Applesauce

Applesauce contains apple, sugar, and other sweeteners, honey, acidulants, salt, flavorings, spices, preservatives, etc. The desired characteristics of applesauce are golden creamy color, a balance of sweetness and tartness, glossy texture, which is not soft and mushy. The Brix of unsweetened applesauce is about 9.0. However, the Brix of sweetened applesauce is about 16.0. Applesauce has a pH of approximately 3.4–4.0. The basic processing steps involved are: dice/chop washed apples (a blend of several apple cultivars, in peeled or unpeeled forms can be used) → heat process apple pieces (similar to blanching, this step is critical for consistency and texture of the finished product) → pass through a pulper/finisher (screen size: 0.16–0.32 cm; for coarse grainy sauce: 0.25–0.32 cm) to remove seeds, peels, etc. → fill and seal in containers (the fill weight is 90% of the container's capacity and allow for a head space of about 0.6 cm upon cooling) → heat process (if the fill temp is ≤88°C, heat containers in boiling water for 10–15 min in an open container to insure microbiological safety; a high-pressure processing ensures product safety and longer shelf life by destroying spoilage organisms) → invert containers and cool.

34.6.4 Processed Frozen Apples

Apple can be infused and pasteurized for use in frozen desserts such as ice cream, frozen yogurt, etc., or for addition into baked goods. The water content of apple is stabilized by infusion with sweeteners or by incorporating stabilizer (pectins, carrageenan, gums, and starches). The fruits thus processed are not icy when frozen. The products are pasteurized and are a ready to use ingredients in frozen desserts which cannot use IQF or frozen fruits. The process maintains fruit piece identity, natural color, texture, and flavor (Sinha 1998).

34.6.5 Dried Apples

The drying of apples at a given temperature would depend on raw material (fresh or frozen, with or without skin), composition, size and geometry (sliced, diced, etc.), and drying load. In general, drying rate follows three phases (1) a initial phase of rapid water evaporation, because the fruit contains relatively large amounts of unbound moisture; (2) a steady evaporation phase thereafter; and (3) a falling and slow rate of evaporation towards the end because the water is more tightly bound to the fruit constituents and is difficult to dislodge.

The rate and completion of drying can be monitored by measuring changes in water activity (a_w). The cut-off a_w of shelf stable dried fruits should be below 0.65; above which mold can grow (Beuchat 1981), unless antimycotic agents are used.

Consumers like the dried apples to be as close as possible in eating quality to a fresh apple. Thus the texture, color, and taste of dried apples are important. The aim of infusion drying as discussed below is precisely to achieve this. Most dried foods with reduced moisture are partly or completely amorphous. According to the glass transition (T_g)

theory, a glass (amorphous material) is changed into a super-cooled melt or liquid during heating, or to reverse during cooling. Mobility of water is high in glassy food systems. Following completion of drying, the apples should be equilibrated at cooler temperatures to enable a free-flowable texture.

In dried fruits, plasticization (T_g decreases) is mainly due to water, but other solutes such as glycerin may also act as plasticizers to enable soft textured dried products (Le Meste and others 2002). The T_g of freeze-dried apples has been reported to be $33.8 \pm 3.4^{\circ}C$. The T_g is shown to increase with the temperature of drying. The T_g represents the thermal limit between a state where the molecular mobility is rather low (no reaction) and a state of increased molecular mobility, which favors diffusion and other reactions. Thus a freeze-dried strawberry with a T_g of $45.4 \pm 2.2^{\circ}C$ would be less

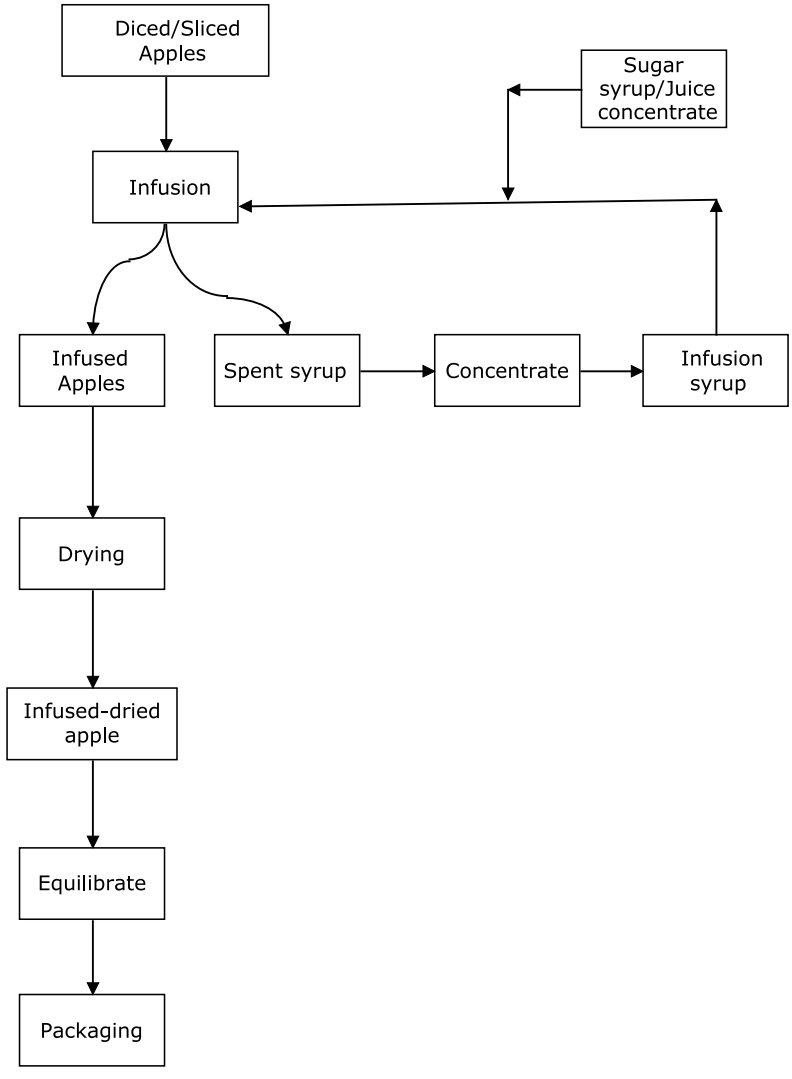

Figure 34.2 *Process for infused-dried apples.*

susceptible to color changes than freeze dried apples (T_g of 33.8 $\pm$ 3.4°C) or pear (T_g of 24 $\pm$ 2.6°C). However, other factors (besides T_g) such as, internal fruit structure, porosity, thermal conductivity, matrix elasticity, temperature within the product, and rate of heat transfers during drying can affect fruit quality (Khalloufi and Ratti 2003).

The hygroscopic properties of dried apple chips are due to their composition of sugars and pectins, in combination with their porous structure. At a_w below 0.12 apple chips demonstrated excellent crispness (Konopacka and others 2002).

During air-drying of apple rings, nonuniform moisture and/or temperature distribution can cause varying degree of shrinkage. Apples dried at 60–65°C showed higher cellular collapse than those dried at 40–45°C and 20–25°C (Bai and others 2002).

Infused dried apples retain their shape and texture much better than those, which are traditionally dried. A range of texture from chewy to crisp can be achieved by controlling the water activity of infused dried apples. The infusion drying process (Fig. 34.2) consists of infusing diced or sliced apples in a sweetener or juice solution to a desired Brix range, separating it from the infusion solution and drying to generally about 0.40–0.60 water activity (about 8–14% moisture). The spent syrup is concentrated and recycled.

Infused-dried apple is a value-added ingredient in various foods or snacks. Unlike other dried apples, infused-dried apples can be diced for application into foods. With infusion-drying use of sulfites is not warranted (Sinha 1998). On the basis of about 40 g serving size, the infused dried apples are not high in calories (Table 34.14), besides noncaloric sweeteners can be used for infusion. Infusion process can allow incorporation of desirable vitamins, minerals, flavors, and colors as well.

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35

Strawberries and Blueberries: Phytonutrients and Products

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35.1 Strawberries 794
35.1.1 Introduction 794
35.1.2 Supply and Demand 794
35.1.2.1 Supply 794
35.1.2.2 Demand 795
35.1.3 Strawberry Varieties and Postharvest Handling 795
35.1.3.1 Strawberry Variety 795
35.1.3.2 Postharvest Handling 795
35.1.4 Physico-chemical, Phytonutrients, and Nutritional Qualities 796
35.1.4.1 Sugars, Organic Acids 796
35.1.4.2 Pectins and Cell Wall Enzymes 797
35.1.4.3 Strawberry Flavor 797
35.1.4.4 Phytonutrients 798
35.1.4.5 Nutritional Quality 800
35.1.5 Strawberry Products 800
35.1.5.1 Frozen Strawberries 800
35.1.5.2 Strawberry Puree 800
35.1.5.3 Strawberry Juice 801
35.1.5.4 Infused and Stabilized Frozen Strawberries 803
35.1.5.5 Dried Strawberries 804

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

35.2 Blueberries 805
35.2.1 Introduction 805
35.2.2 Supply and Demand 805
35.2.2.1 Supply 805
35.2.2.2 Demand 807
35.2.3 Physico-chemical, Phytonutrients, and Nutritional Qualities 808
35.2.3.1 Physicochemical Quality 808
35.2.3.2 Flavor Compounds in Blueberries 808
35.2.3.3 Phytonutrient Quality 808
35.2.3.4 Nutritional Quality 811
35.2.4 Blueberry Products 811
35.2.4.1 IQF Blueberries 811
35.2.4.2 Blueberry Puree 811
35.2.4.3 Blueberry Juice and Concentrate 811
35.2.4.4 Infused and Stabilized Frozen Blueberries 811
35.2.4.5 Infused-Dried Blueberries 811
35.2.4.6 Freeze-Dried and Vacuum Dried Blueberries 811
References 812

35.1 STRAWBERRIES

35.1.1 Introduction

The strawberry (Fragaria x ananassa Dutch) is prized for its typical red color, heart-like shape, and fresh eating qualities (small size, soft skin, and fresh fruit flavor). Strawberries used in products such as ice-cream, yogurt, bakery products, ready-to-eat cereals, breakfast bars, and so on, enhance consumer value in these products. However, strawberries are one of the few important commercial fruits to be grown close to the soil, which leads to harvesting and microbial challenges. Development of disease-resistant strawberry varieties, soil fumigation, hill plasticulture, and drip irrigation have positively impacted the strawberry industry. Further, recent investigations into the beneficial effects of bioactive phenolic compounds in berries are likely to boost demand for strawberries. This chapter provides an overview of supply and demand, physico-chemical, phytonutrients, nutritional, and processing aspects of strawberries.

35.1.2 Supply and Demand

35.1.2.1 Supply. The average production of strawberries in the world during the period 2001–2005 was about 3.4 million metric tons (Table 35.1). The top ten producing countries accounted for 65% of harvested acreages, and 75% of world production. The United States is the leading producer (27% of world production), and more than 80% of these strawberries are grown in the state of California. The farm gate value of strawberries ($1.47 billion) in the United States was close to oranges ($1.57 billion) in 2004 (USDA 2005). Poland and Russia have the largest areas under strawberry cultivation, but the per acre yield is low. In China strawberries are cultivated in greenhouses, plastic sheds, and open fields. China is emerging as a major producer and exporter of strawberries to other countries, including the United States and Japan.

TABLE 35.1 Strawberry Production in Leading Countries: Average for 2001–2005.

Countries	Average Production (2001–2005; Metric Ton)	Average Area Harvested (2001–2005; Hectare)
1. United States	912,070	19,705
2. Spain	290,771	8,605
3. S. Korea	204,166	7,443
4. Japan	204,040	7,326
5. Russia	202,400	33,520
6. Poland	191,707	50,747
7. Italy	162,458	6,368
8. Turkey	145,400	10,200
9. Mexico	144,743	5,558
10. Germany	112,401	10,839
World	3,364,760	243,725

Source: FAO (2006); available at http://faostat.fao.org/faostat

35.1.2.2 Demand. In the United States strawberry is the leading berry, with annual per capita consumption of over 3.0 kg (USDA 2005). About 75% of strawberries produced in the United States are utilized as fresh, and the remaining 25% are frozen and processed into other products. Demand for strawberry is also strong in Japan and many European countries.

35.1.3 Strawberry Varieties and Postharvest Handling

35.1.3.1 Strawberry Variety. Based on flower and fruit-bearing properties, strawberries are grouped as:

1. Short-day or June-bearers, which flower in fall when the days are relatively shorter and bear fruits in the following spring – these strawberries produce a single crop each year;
2. Everbearers, which initiate flowers and fruits during the long days of summer; and
3. Day-neutrals, which initiate flowers under any day length.

In selecting a variety, considerations are given to disease resistance, yield, and end-use requirements. For example, firmness and drip loss are important for strawberries to be used in processing. Table 35.2 gives characteristics of selected strawberry varieties.

35.1.3.2 Postharvest Handling. Strawberries are picked by hand and harvesting is carried out several times during the season because the berries do not ripen simultaneously. Strawberries are very perishable (the market life of fresh strawberries is only 1–2 weeks from harvest) and prompt cooling is important for maintaining fruit quality. Carbon dioxide gas is injected into strawberry crates before shipment to slow respiration, and transportation with refrigerated carriers helps in extending fruit quality. Because strawberries are grown close to the ground, simply washing with water does not ensure food safety. A hepatitis A (a viral liver disease) outbreak occurred in some parts of the United States in 1997 after consumption of sliced

TABLE 35.2 Characteristics of Selected Strawberry Varieties.

Variety	Characteristics
Allstar	A mid-season maturity strawberry of large size; produces high yield; light red exterior and light pink to white interior color; sweet; medium firmness; a good dessert-quality strawberry
Annapolis	Early to mid-season large fruit; light red; soft texture; mild flavor; a good strawberry for fresh consumption and freezing
Aromas	A day-neutral strawberry developed in California with good yield potential; firm with good flavor and red color; suitable for fresh market and processing
Camarosa	An important short-day variety in southern California; long wedge shape, good firmness; uniform red color; fruits vary in sweetness; high acidity; suitable for fresh market and freezing
Cavendish	A good quality and juicy strawberry similar to Annapolis; dark red and glossy
Chandler	A short-day variety; large and firm; suitable for fresh market and processing
Diamante	A day-neutral variety; more vigorous than Selva; requires less chilling to maintain excellent fruit quality; larger fruit than Aromas, lighter in color especially on interior fruit; suitable for fresh market and processing
Senga Sengana	A popular variety of Poland; medium size; good for freezing
Sweet Charlie	A strawberry from the Florida region; distinct sweet flavor; size similar to Selva; external fruit color is orange red, and internal color is orange streaked with white
Totem	A major short-day variety of Pacific northwest; attractive red color; uniform medium shape; firm texture; suitable for fresh market and processing
Ventana	A short-day strawberry from California; bright light red fruit of good quality; production pattern similar to Camarosa

Sources: http://www.msue.msu.edu/vanburen/e-839.htm; http://wwwcalstrawberry.com; http://www.oregon-strawberries.org; http://strawberry.ifas.ufl.edu/breeding/varieties.htm; http://www.gov.on.ca/OMAFRA/english /crop/facts/strawvar.htm

frozen strawberries. However, washing strawberries with chlorinated water was reported to significantly reduce microbial population, hepatitis A, and other viruses (Williamson 1998).

35.1.4 Physico-chemical, Phytonutrients, and Nutritional Qualities

35.1.4.1 Sugars, Organic Acids. Sugars are present naturally in strawberries primarily as, fructose, glucose, and sucrose. However, arabinose, xylose, and inositol have also been reported (Castro and others 2002; Macias-Rodriguez and others 2002). During ripening, as the strawberries turn from half red to dark red, an increase in sugars (sucrose: 1.4% to 1.7%; glucose: 2.2% to 2.9%; fructose: 2.4% to 3.1%) and

TABLE 35.3 Physico-chemical and Sensory Qualities of Selected Strawberries.

				Sensory scores[a]				
Variety	Soluble Solids	Titrable Acidity (% as Citric)	pH	Color	Sweet	Sour	Texture	Overall
Rainier	7.6	1.07	3.32	6.1	4.1	5.0	4.6	4.4
Redcrest	9.7	1.04	3.18	6.5	2.6	3.8	6.6	3.1
Selva	7.8	0.92	3.49	5.2	4.0	3.8	6.1	4.5
Sumas	7.7	0.91	3.24	6.9	4.7	4.4	6.9	4.8
Totem	8.4	1.07	3.35	8.2	4.2	5.0	5.5	4.6

Source: Shamaila and others (1992).
[a]Sensory scores based on a 10-cm scale.

malic acid (0.3% to 0.4%), but a decrease in citric acid (0.7% to 0.5%) have been reported (Menager and others 2004). Strawberries vary in level and types of acids they contain. For example, a study of "Camarosa" and "Selva" strawberries showed citric acid, malic acid, and ascorbic acid contents as: 0.76%, 0.13%, 0.04% and 0.5%, 0.0%, 0.04%, respectively (Castro and others 2002).

Table 35.3 presents physico-chemical and sensory data of selected strawberries. Strawberry varieties analyzed had a Brix range of 7.6–9.7. The overall consumer ratings of these strawberries were dependent on sweetness and odor. Variety Redcrest was sour (lowest pH) and scored least on the overall fruit quality rating. The Totem strawberries scored highest for color by the judges. Azodanlou and others (2003) reported a significant positive correlation ($r = 0.94$, $p \leq 0.05$) between strawberries' °Brix and overall consumer acceptance.

Effect of Growing Conditions on Sugars. Wang and others (2002) reported effects of growing conditions, matted-row (MR, without plastic mulch) vs. hill plasticulture (HC, beds covered with black polyethylene mulch) on sugars and other quality characteristics. The average fructose, glucose, and sucrose contents of 14 strawberry varieties grown on MR and HC were 2.13% and 2.39%, 1.83% and 2.22%, and 0.73% and 0.73%, respectively.

35.1.4.2 Pectins and Cell Wall Enzymes. Lefever and others (2004) reported that soft strawberries have more water-soluble pectin, and higher pectin methylesterase (PME) and polygalacturonase (PG) activities than firm strawberries. PME and PG are considered primary cell wall hydrolases involved in fruit softening. Darsanga strawberries, which had lower levels of water-soluble pectins and hydrolases, were firmer and had 50% less drip loss than Senga Sengana. Postharvest dipping of strawberries in 1% calcium chloride followed by storage at 1°C for 1 day was shown to improve firmness of strawberries (Garcia and others 1996).

35.1.4.3 Strawberry Flavor. Naturally present sugars in strawberries are important nonvolatile flavor components. An increase in sugars in strawberries during maturity and ripening produces strawberry aromas (Bood and Zabetakis 2002). The typical strawberry flavor is due to esters, alcohols, and carbonyl compounds. Table 35.4 lists important aroma compounds in strawberries. The presence of esters such as acetates, butanoates, and

TABLE 35.4 Important Flavor Compounds of Strawberries.

Compounds	Flavor Notes
Methyl butanoate	Fruity (fresh strawberry)
Ethyl butanoate	Fruity
Methyl hexanoate	Fruity, pineapple
Hexyl acetate	Banana, apple, pear
Hexanal	Green, sour, cut grass
Trans-2-hexenol	Green leaves
Linalool	Lemon peel, flower
2,5-Dimethyl-4-methoxy-3(2H)-furanone	Caramel-like, burnt
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	Caramel-like, sweet
1-octanol	Chemical

Sources: Azodanlou and others (2003), Perez and Sanz (2001), Schieberle and Hofmann (1997).

hexanoates give fresh, fruity, strawberry aromas. Hexanal and trans-2-hexenol give green cut grass notes. Furaneol: 2,5-dimethyl-4-hydroxy-3 (2H)-furanone (DHF), and mesifuran: 2,5-dimethyl-4-methoxy-3 (2H)-furanone (DMF) together with other flavor compounds contribute to the overall strawberry flavor. Until strawberries turn about half red, furanones and esters are not detected; at full maturity more furaneol and mesifurane than other flavor compounds are analyzed (Menager and others 2004). Both DMF and DHF were shown to correlate with fresh strawberry flavor (Golaszewski and others 1998).

35.1.4.4 Phytonutrients

Anthocyanins. The typical red strawberry color is due to anthocyanins, pelargonidin 3-glucoside (P 3-G; orange color), cyaniding 3-glucoside (C 3-G; red color), pelargonidin 3-rutinoside and pelargonidin 3-glucoside succinate (Garzon and Wrolstad 2002; Wang and others 2002; Kosar and others 2004). Differences in anthocyanin concentration (and thus the strawberry color) can occur as a result of cultivation practices, harvest maturity, variety, geographical location, storage, and processing. For example, strawberries grown on hill plasticulture (HC) and flat matted row (MR) had average P 3-G levels of 55.4 mg and 51.5 mg per 100 g fresh weight, and average C 3-G levels of 3.6 mg and 2.8 mg per 100 g fresh weight, respectively (Wang and others 2002). Aaby and others (2005) reported that although the major anthocyanin in the strawberry flesh was P 3-G, achenes (seeds) attached to the strawberry surface consisted of equal amounts of C 3-G and P 3-G.

Freezing strawberries causes minimal loss of anthocyanins (Zabetakis and others 2000). Often, sugar is added (Wrolstad and others 1990) to strawberries before freezing to minimize color loss, and sugar-pack frozen strawberries are softer and easier to handle.

Storage of strawberries in various CO_2 atmospheres has little effect on the anthocyanin content of the exterior tissues, but causes anthocyanin loss in the interior tissues (Gil and others 1997).

Maintaining strawberry color during storage and processing is difficult. Exposures to light, oxygen, high pH, and high temperature have negative effects on anthocyanins. For example, during concentration about 22% loss of anthocyanin occurred and the anthocyanins content of strawberry juice (8 Brix) and concentrate (68 Brix) were 26.8 mg to 29.0 mg, and 21.0 mg to 22.7 mg per 100 mL, respectively (Garzon and Wrolstad 2002).

A study by Wesche-ebeling and Montgomery (1990) of the effect of strawberry polyphenol oxidase (PPO) on D-catechin alone or in combination with pelargonin or cyanin showed formation of brown pigment. However, little PPO activity was observed with pelargonin and cyanin as substrates. The PPO's effect on D-catechin was responsible for 50–60% loss of the anthocyanins after 24 h at room temperature. Rwabahizi and Wrolstad (1988) have suggested that a thermal loss of anthocyanins and not enzymatic oxidation causes browning of strawberry juice.

Addition of ascorbic acid (which inactivates PPO) had negative effect on color of strawberry syrup (Skrede and others 1992). It is likely that the hydrogen peroxide formed as a result of ascorbic acid degradation affects anthocyanins.

Phenolic Compounds. Strawberry contains many phenolic compounds with potential health benefits. The concentration of total phenolics in 14 strawberries has been reported to be 70.5 mg to 80.3 mg/100 g fresh weight. Important phenolic compounds (based on 100 g fresh weight) in strawberries are ellagic acid (0.81–0.96 mg), ellagic acid glucoside (0.96–1.24 mg), quercetin 3-glucoside and quercetin 3-glucuronide (1.65–2.12 mg), and kaempferol 3-glucoside (0.31–0.36 mg) (Wang and others 2002).

Antioxidant Capacity. Anthocyanins, phenolic acids, and flavonoids are sources of natural antioxidants in our diet. On a dry weight basis, strawberries (147.7 μmol TE/g) had slightly more oxygen radical absorbance capacity (ORAC) than black raspberries (136.2 μmol TE/g), blackberries (133.3 μmol TE/g), and red raspberries (104.3 μmol TE/g) (Wang and Lin 2000). In a more recent study, water-soluble and lipid-soluble antioxidant capacities in market samples of strawberries were shown to be 35.41 μmol TE/g and 0.36 μmol TE/g (as is basis; % moisture = 91.1), respectively (Wu and others 2004).

Antiproliferative Properties. The phytochemicals in strawberry extracts have been shown to inhibit proliferation of $HepG_2$ human liver cancer cell (Meyers and others 2003). However, the antiproliferative activities did not correlate with their antioxidant activities ($r = 0.41$, $p > 0.05$) or total phenolics ($r = 0.37$, $p > 0.05$). It is likely that a specific phenolic compound or classes of phenolics in fruits are responsible for antiproliferative activities (Sun and others 2002).

TABLE 35.5 Nutrients in Strawberries.

Nutrients/100 g	Fresh Strawberry[a]	Frozen Strawberry[a]	Strawberry Juice[a]	Infused-Dried Strawberry[b]
Calories (kcal)	32.0	35.0	30.0	325.0
Total fat (g)	0.30	0.11	0.40	1.04
Sodium (mg)	1.0	2.0	1.0	25.0
Potassium (mg)	153.0	148.0	166.0	382.0
Total carbohydrate (g)	7.68	9.13	7.00	82.20
Total fiber (g)	2.0	2.10	0.10	10.20
Soluble fiber (g)	0.80	0.65	0.03	3.80
Insoluble fiber (g)	1.20	1.45	0.07	6.40
Sugars (g)	4.66	6.96	6.90	70.30
Protein (g)	0.67	0.43	0.60	3.16
Calcium (mg)	16.0	16.0	14.00	160.0
Vitamin C (mg)	58.8	41.20	28.40	95.0
Vitamin A (IU)	12.0	45.0	20.0	41.0
Water (g)	90.95	90.0	91.60	12.0

[a]*Source*: USDA: http://www.nal.usda.gov/fnic/foodcomp/search/
[b]Courtesy Graceland Fruit Inc, Frankfort, Michigan, USA.

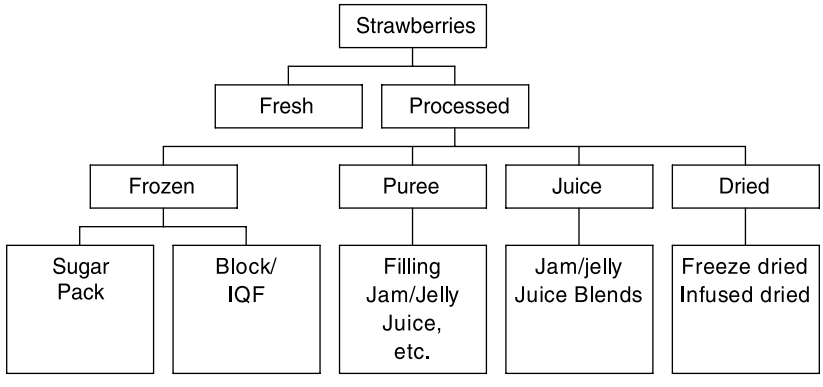

Figure 35.1 *Utilization of strawberries as products.*

35.1.4.5 Nutritional Quality. Fresh strawberries are a source of fiber, potassium, and vitamin C. The sugar infused-dried strawberries, which are used as snacks and ingredients in various foods, have <100 calories per 28 g serving size. These products are also a good source of dietary fiber (Table 35.5).

35.1.5 Strawberry Products

Figure 35.1 shows utilization of strawberries as products. Strawberry is frozen or made into a puree. The processing steps for strawberry products are similar to other fruits. However, specific value-added products have been developed, for example, a light crispy freeze-dried strawberry for use in ready-to-eat cereals and strawberry fruit preparations for ice-creams and yogurts. This section highlights processing of selected strawberry products.

35.1.5.1 Frozen Strawberries.

IQF and Block Frozen Strawberries. Frozen strawberries can be used as such or as raw materials for a variety of products. For example, individual quick frozen (IQF) strawberries are used for freeze-drying and infusion drying. The IQF strawberries can also be sliced or diced for use in ice-creams, jelly, and jam. It is important to select uniform red color and good quality fruits for freezing. The process consists of:

Fruit reception $\longrightarrow$ precooling ($\sim 0-2^{\circ}$C) to remove field heat $\longrightarrow$ removal of stems and caps $\longrightarrow$ air classification to remove extraneous matter $\longrightarrow$ inspection and grading $\longrightarrow$ washing with chlorinated (20–35 ppm) cold water $\longrightarrow$ individually quick freezing the fruit (about -40°C) in a blast air freeze tunnel $\longrightarrow$ metal detection $\longrightarrow$ packaging and storage under frozen temperatures (-18°C).

Quick freezing reduces formation of large ice crystals, which cause drip losses upon thawing. The IQF strawberries have free-flowing texture and hold their color and shape better than block frozen or sugar-pack strawberries. Frozen strawberries were shown to contain higher ascorbic acid and total phenolics than dried strawberries (Asami and others 2003). Often, freezer burn (off-flavor and discoloration) due to moisture loss or ice crystal evaporation from fruit surface is encountered in improperly packaged strawberries held in freezers for an extended period. Packaging in heavyweight, moisture-resistant, and airtight packages is helpful in minimizing this problem.

Sugar Pack Frozen Strawberries. In this process, sugar is added on top of the strawberries, and the product is frozen below -18°C. In the industry, $4+1$ (80% fruit $+20\%$ sugar) and $7+3$ (70% fruit plus 30% sugar) pack strawberries are common. These products are used in fillings, baking, dairy, and other applications.

35.1.5.2 Strawberry Puree. Fruits, not sold as fresh or frozen, can be made into puree or pulp. It is critical that moldy and rotten fruits are not used for processing into puree. The fruit preparation steps (cleaning, sorting, and washing with chlorine) are as previously described. However, it is not necessary to remove the berry cap. Figure 35.2 shows the basic steps involved in making strawberry puree. A pulper with screens (0.5–10 mm) mashes the fruit into a smooth pulp or puree and also removes, skins, seeds, and so on. Depending on the size of the screens used, the resulting puree would be either seedless or contain some seeds. Often the puree is passed through a vacuum system to maintain flavor and color and pasteurized (88°C for ~ 2 min and cooled to about 15°C). The finished

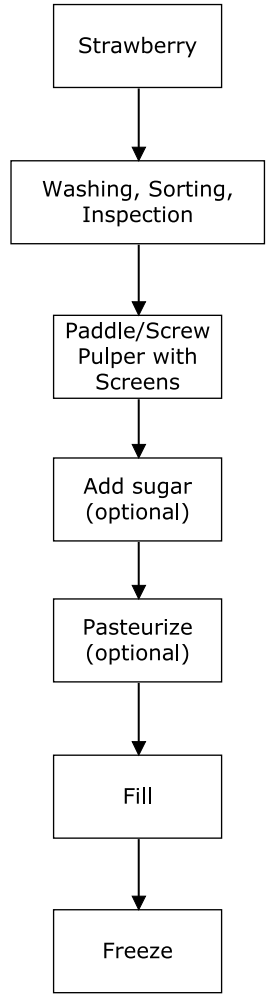

Figure 35.2 *Basic steps in making strawberry puree.*

product is metal-detected and stored frozen. A single strength strawberry puree will have the same °Brix as the starting raw fruit. However, concentrated strawberry purees of about 28°Brix are also available. The puree may be treated with enzymes and filtered before concentration to provide better quality puree for use in jams, juices, and so on. Sweeteners such as sucrose can also be added to the puree to adjust the °Brix depending on the end use.

35.1.5.3 Strawberry Juice. Strawberry juice is mostly used in fruit juice blends. Figure 35.3 describes the steps involved in strawberry juice manufacture. Following pressing/centrifugation to obtain the juice, pectinase enzymes (e.g., Rapidase® Super BE @ 3 mL/kg; 50°C for 2 h) can be used for clarification. Subsequently, a clear juice is obtained through use of membrane separation processes. A single strength pasteurized (88°C for 1 min followed by cooling to 2°C) or concentrated (to higher °Brix) juice can be made.

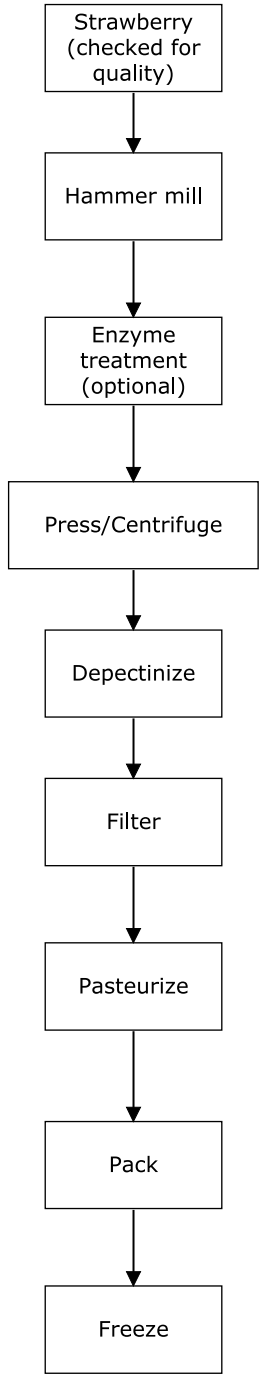

Figure 35.3 *Steps in making strawberry juice.*

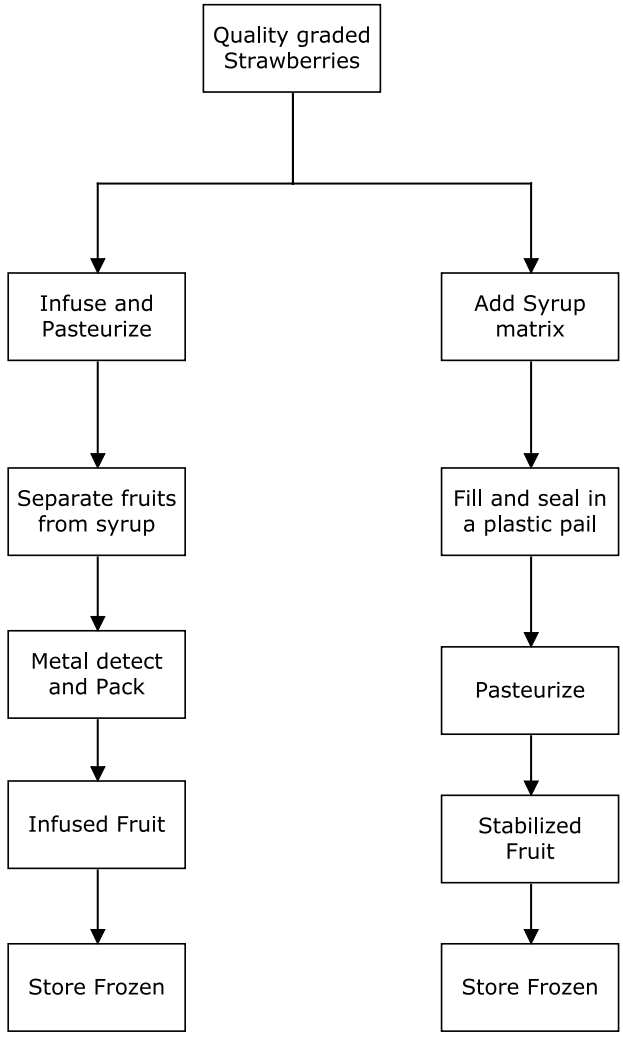

Figure 35.4 *Process for making infused and stabilized frozen strawberries.*

35.1.5.4 Infused and Stabilized Frozen Strawberries. Figure 35.4 shows the process for infused and stabilized frozen strawberries. The process for infused strawberry consists of infusing whole or sliced strawberries in sugar solution or corn syrup or fruit juice to about 25–45°Brix depending on application, processing (heating at least to 65°C with 5 min hold and cooling to about 24°C), separating the processed strawberries from the infusion solution, metal detecting, packaging, labeling, and storing at −18°C. The process for stabilized frozen whole or sliced strawberries consists of combining about 50–70% fruits with a syrup matrix (30–50%), which contains sweeteners (sugar, corn syrup, fruit juice), stabilizers (pectins, starch, carrageenan, gums), acidulants (citric acid, malic acid, ascorbic acid), and flavorings, in-pail processing (preferable in a retort) with agitation at 80–82°C with 5 min hold time, and cooling to 20–24°C. The stabilized strawberries are stored frozen (−18°C) until used. Both infused and stabilized

strawberries are soft (unlike IQF and block frozen strawberries) even when frozen; the fruit piece identity, natural, color, and flavor of the fruit can be retained without use of a preservative such as potassium metabisulfite (Sinha 1998). These products have excellent microbial quality and can be used directly without further processing in ice cream, sorbets, yogurts, frozen bakery products, and so on.

35.1.5.5 Dried Strawberries

Freeze Dried Strawberry. Dried strawberries are used in ready-to-eat cereals, dry bake mixes, breakfast or other food bars, as snacks, and so on. Although traditional atmospheric drying provides a higher drying rate than freeze-drying or vacuum drying, the quality of dried strawberries is not very appealing. There is a loss of the red strawberry color and the texture is hard. Freeze-drying provides perhaps the best retention of natural red strawberry color and shape. Further, it has a positive effect on retention of ascorbic acid and phenolics (Asami and others 2003). However, freeze-drying is expensive and time-consuming (about 1 day or more compared with a few hours for atmospheric drying).

IQF whole or sliced strawberries work well as raw materials for freeze-drying that takes place under vacuum. The pressure in the drying chamber is typically (boiling point of water is $\leq 10^{\circ}$C), where water is transformed from the ice state to water vapor and removed through sublimation. The sublimation temperature for strawberries is -15°C (Khalloufi and Ratti 2003). As a majority of water is removed at a very low temperature, color and shape are maintained. Subsequently, to complete drying and reduce the moisture of the strawberries to about 2%, the temperature of the heating plates can be raised from 20°C to 50°C, while maintaining a low vacuum pressure. Crushing strawberries before drying and low drying temperatures ($<40^{\circ}$C) has a positive effect on strawberry flavor. However, off-flavor was detected when the temperature of the heating plates was raised to 60°C (Paakkonen and Mattila 1991).

Freeze-dried strawberries have a light, crisp, porous texture and their low bulk density (about 0.1 g/cc) and water activity (about 0.2) makes them ideal for use in ready-to-eat cereals. However, freeze-dried strawberries require special laminated packaging to maintain a crisp texture.

Infused-Dried Strawberries. In this process the strawberries are infused in a sugar or juice solution to increase the initial Brix content by three-fold or more and simultaneously decrease its moisture. This moisture loss from the fruit takes place at a relatively lower temperature than in the drying process. The fruit surface serves as a semipermeable membrane for exchange of sugars and water to and from the infusion medium. A number of variables beginning with fruit itself (fresh or frozen, initial Brix, size, whole, sliced, or diced), infusion syrup's Brix, fruit to syrup ratio, infusion temperature, infusion time, agitation, and type of infusion vessel influence infusion process. After infusion, the initial moisture of the fruit can be reduced by 30–40%. The infused strawberries are separated from the infusion syrup and dried using traditional dryers. The moisture of the infused-dried strawberry can range from 8% to 14% (water activity, <0.60). These product are soft and can be diced into julienne pieces for use in other foods (Sinha 1998). The flavor of infused-dried strawberry is good (Escriche and others 2000). However, there can be some loss of strawberry color, especially if the drying temperature is too high. The product is shelf stable for one year or more.

35.2 BLUEBERRIES

35.2.1 Introduction

Blueberries (family, Ericaceae; genus, Vaccinium) are indigenous to North America. The Vaccinium genus consists of many species, which have edible fruits. Like bilberry (V. myrtillus), cranberry (V. macrocarpon), lingonberry (V. oxycoccus), and huckleberry (V. ovatum and V. vaccillans), blueberries are valued fruits. Native Americans enjoyed fresh blueberries and also dried them for use with other foods. They are believed to use blueberry roots and leaves as medicine. A tea made from blueberry leaves was considered good for blood and blueberry juice was used to treat coughs (Anon 2004). These early insights and experiences about the health benefits of blueberries are now being corroborated. Ehlenfeldt and Prior (2001) showed that blueberry leaves have higher phenolics and antioxidant values than the fruit tissues. In laboratory studies, dried extract of blueberries having high antioxidants reversed signs of aging (Joseph and others 1999). Other studies (Schmidt and others 2004; Kraft and others 2005) have shown that specific wild blueberry extracts may have antiadhesion (against *E. coli*, which causes urinary tract infection) and chemopreventive properties. Thus, blueberries are emerging as a functional fruit for improving health and quality of life. This section provides an overview of supply, demand, physico-chemical, phytonutrient, nutritional, and processing aspects of blueberries.

35.2.2 Supply and Demand

35.2.2.1 Supply. The production of blueberries is currently concentrated in North America. The United States and Canada produce more than 80% of the blueberries harvested throughout the world and also lead in advancing the blueberry as a commercial fruit. Outside North America, Poland is the largest producer, with about 10% of the world total (Table 35.6). Argentina and Chile have also begun commercial production of blueberries and import to the United States. The types of blueberries commercially harvested in the United States and Canada are:

1. Northern highbush or cultivated blueberries (V. corymbosum);
2. Southern highbush blueberry (V. virgatum);
3. Rabbiteye blueberries (V. ashei);
4. Lowbush or wild blueberries (V. angustifolium).

TABLE 35.6 Leading Blueberry Producers in the World.

Countries	Average Production (2001–2005; metric Ton)	Average Area Harvested (2001–2005; Hectare)
United States	121,415	17,028
Canada	74,448	25,057
Poland	21,080	3,900
Ukraine	4,180	680
Netherlands	3,960	980
Romania	3,700	580
Lithuania	2,280	580
New Zealand	1,700	370
Italy	1,568	195
World	237,199	49,880

Source: FAO (2006).

TABLE 35.7 Characteristics of Selected Blueberry Varieties.

	Characteristics
	I. Northern Highbush (Cultivated Blueberries)
Bluecrop	Leading commercial variety in North America; ripens mid-season; very productive; bears fruit over one-month harvest season; large, light blue; small scar; firm; mild tart flavor; good for fresh market and processing
Duke	An early variety; frost-tolerant; medium size firm fruit; medium blue color with a small scar; good flavor; suitable for IQF and fresh shipping
Elliott	A late season berry; light blue; medium size; firm; small scar; mild tart flavor; suitable for fresh market and processing
Jersey	One of the earliest (1928) released late season variety; small firm fruit with medium scar; light blue; suitable for processing
Nelson	A late season berry; large, light blue, firm; good quality fruit for fresh use and processing
	II. Southern Highbush
O'Neal Reveille	A very low chill requirement variety; large size berry; mild sweet flavor
	III. Rabbiteye
Brightwell	An early season fruit; medium size; blue color, gritty texture and tough skin
Premier	An early season fruit; large fruit with good flavor
Tifblue	A benchmark for rabbiteye; mid season variety; large fruit
Powderblue	Similar to Tifblue but slightly smaller; blue color with slight white powdery coat, thus the name; resist cracking from rain
	IV. Lowbush (Wild Blueberries)
Cumberland	High in soluble solids and dry matter; not as firm
Fundy	Low dry matter and soluble solids content; high in acidity and anthocyanins

Sources: Blueberry Production Summary—Cornell Cooperative Extension 2002, available at http://www.cce.cornell.edu; Jim Hancock 2001: Blueberry varieties evaluated, available at http://mtvernon.wsu.edu/frt_hort/blueberry.htm; Kalt and McDonald (1996); Blueberry Information from Michigan State University Extension, Available at http://www.msue.msu.edu/fruit/bbvarbul.htm; Blueberry Information from University of California Cooperative Extension, available at http://www.mastergardeners.org/picks/bluvar.html

The first three types of blueberries commercially cultivated and harvested are termed "cultivated" blueberries. The lowbush or "wild" blueberries are managed "wild" stands of blueberry. Table 35.7 lists selected blueberry varieties and their characteristics.

The northern highbush blueberries (woody shrubs that can grow to exceed 10 ft but plants are kept pruned to 5–6 ft in height) are commercially planted in cooler regions of Michigan, New Jersey, New York, Washington, Oregon, British Columbia (Canada), and so on. These berries generally require 160 frost-free days; severe winter temperatures (about -20°C or below) will injure most highbush varieties. Southern highbush blueberries with low chill tolerance are grown in southern states. Some traits like bloom date and ripening period of these berries are similar to the northern highbush blueberries (USDA 2003).

Rabbiteye blueberries (Fig. 35.5) are grown in the warmer regions of the southeastern United States. These berries (comparable to highbush) are not winter-hardy, but are generally drought-resistant. They have a somewhat fibrous mouthfeel.

Figure 35.5 *Blueberry Plant (Courtesy of Tim Kelly, Solo Foods, North Carolina).*

Wild blueberry plants (about 1–2 ft tall) are not commercially planted, but natural wild blueberry clones are maintained (pruned, sprayed, and harvested) as a commercial crop in the northeastern United States (primarily Maine) and eastern Canada (Prince Edward Island, New Brunswick, Quebec). The wild blueberry fruits are distinctly smaller than the cultivated blueberries.

In the United States, Michigan leads in cultivated blueberry production, followed by New Jersey, North Carolina, Oregon, Georgia, and Washington. Maine is the largest producer of wild blueberries. The blueberry production in the United States, which was valued at a little over $100 million in 1993 increased to over $325 million in 2004 (USDA 2005).

Blueberries are a perennial crop that can produce for more than 20 years. The berries do not ripen at the same time, therefore harvesting is done several times during the season (USDA 2003). Generally, plant shakers are used for harvesting cultivated blueberries. The harvested berries are kept in plastic crates and stored in cold rooms (at about 2°C) to maintain quality.

35.2.2.2 Demand. The demand for blueberries in the United States and many parts of the world, especially in Japan and Europe, is continuing to grow. In the United States, the consumption of both fresh and frozen blueberries, which was about 0.2 lb each during the 1980s, has almost doubled. A little over 40% of the cultivated blueberries are consumed fresh and the remaining are processed into products. However, in North Carolina and New Jersey, more than 70% of blueberries are sold for fresh consumption. In contrast to

TABLE 35.8 Quality Profile of Blueberries.

Component	Highbush	Lowbush	Rabbiteye
Brix	12.5	10.0	15.0
Dry matter (%)	14.0	15.0	18.0
pH	3.0		3.2
% Titrable acidity (citric)	0.7	0.4	0.5
Average weight (g/berry)	2.3	0.3	2.2
% Sucrose	ND	0.4	NA
% Glucose	7.5	5.0	NA
% Fructose	7.7	5.7	NA

Source: Adapted from Makus and Morris (1993); Kalt and McDonald (1996). Sugar data on IQF blueberries courtesy of Graceland Fruit Inc, Frankfort, MI 49635, USA.
ND: not detected; NA: not available.

cultivated blueberries, most of the wild blueberries produced are processed (USDA 2003). Blueberries are utilized as jam/jellies, juice/concentrates, infused, dried, and so on.

35.2.3 Physico-chemical, Phytonutrients, and Nutritional Qualities

35.2.3.1 Physicochemical Quality. Blueberries have waxy skin, and unlike some other berries (strawberry, raspberry, and cranberry), they are mildly sweet. Table 35.8 shows typical quality profiles of blueberries. Variations in these quality attributes can occur due to variety, maturity, time of harvest, geographic locations, season, storage, and processing. In general, wild blueberries have lower Brix (10–12°) and titrable acidity (0.4–0.7% as citric) than cultivated (11–14°; 0.5–1.3%) blueberries. Wild blueberries contain citric acid, malic acid, and quinic acid, and both wild and cultivated blueberries contain slightly more fructose than glucose (Sapers and others 1984; Kalt and McDonald 1996). The blue color of blueberries is easily denoted by the "–b" value on the Hunter color scale (Simon and others 1996).

35.2.3.2 Flavor Compounds in Blueberries. Blueberries emit a low level of aromatic note relative to other fruits. In the manufacture of concentrated blueberry juice, volatile blueberry essence is recovered and blended back to provide the desired blueberry flavor note. The twelve major volatile constituents analyzed in blueberries are E-hept-3-enal; 2,3,5-trimethylheptane; 1,8-cineole; 2,4-dimethyl-5-ethyl-hept-2-ene; nonanal; camphor; menthone; 2,6-dimethoxyphenol; decanoic acid; α-cubebene; valencene; and hexadec-4-en-6-yne (Simon and others 1996).

35.2.3.3 Phytonutrient Quality

Anthocyanins, Total Phenolics, and Antioxidant Capacity. The typical blue color of blueberries is due to anthocyanin pigments. In highbush blueberry, anthocyanins were analyzed as malvidin (purple color), delphinidin (blue-violet), cyanidin (red), and petunidin (blue-purple) (Zheng and others 2003). Blueberries contain about 2 mg/g fresh weight anthocyanins, and 7 mg/g fresh weight of total phenolics. The total antioxidant capacity ranges from 62 to 110 μmol Trolox equivalent/g fresh weight (Table 35.9).

In an early work (Prior and others 1998), anthocyanins (as cyanidin 3-glucoside/g fresh weight) in northern highbush, southern highbush, rabbiteye, and wild blueberries were quantified as 1.29 mg, 0.93 mg, 1.24 mg, and 0.95 mg, phenolics (as gallic acid equivalent/g fresh weight) as 2.61 mg, 2.27 mg, 3.40 mg, and 2.91 mg, and antioxidant capacity

TABLE 35.9 Anthocyanins, Total Phenolics and Antioxidant Capacity of Blueberries.

Component	Highbush	Lowbush	Rabbiteye	References
Anthocyanins as cyanidin 3-glucoside (mg/g as is basis)	2.08 ± 0.78	2.08 ± 0.04	2.42 ± 0.06*	*Variety Bluegem; Moyer and others (2002)
Total phenolics as gallic acid (mg/g as is basis)	5.31 ± 0.96	7.95	7.17 ± 0.016*	Wu and others (2004); *Rabbiteye variety Bluegem from Moyer and others (2002)
Total antioxidant capacity (μmol TE/g as is basis)	62.20	92.60	110.8*	Wu and others (2004); *Rabbiteye variety Bluegem from Moyer and others (2002)

(oxygen radical absorbance capacity or ORAC measured as Trolox equivalent/g fresh weight) as 24.0 μmol, 16.8 μmol, 25.0 μmol, and 25.9 μmol, respectively. These researchers showed a higher linear relationship between total phenolics and ORAC value ($r_{xy} = 0.85$) than anthocyanins and ORAC value ($r_{xy} = 0.77$). Kalt and others (2001) reported more anthocyanins and phenolics in wild blueberries (1.63 mg/g and 3.76 mg/g on a fresh weight basis, respectively) than highbush (1.18 mg/g and 1.91 mg/g) blueberries. Their analysis also showed higher antioxidant values (ORAC) in wild blueberries (69.8 μmol/g fresh weight) compared to the highbush (45.2 μmol) blueberries. Other studies (Moyer and others 2002; Wu and others 2004) have also shown higher phytonutrients (especially, phenolics and antioxidants) in rabbiteye and wild blueberries than the cultivated blueberries (Table 35.9).

Effect of Processing on Phytonutrient Quality. Skrede and others (2000) analyzed anthocyanin concentration in pasteurized (single-strength highbush blueberry juice of 15.0 Brix) and concentrated (Brix 73.5) juice as 0.38 mg/g and 1.78 mg/g, respectively. The recoveries for anthocyanins, flavonol, procyanidins, and chlorogenic acid in the single-strength juice were 32%, 35%, 43%, and 53%, respectively. The press-cake

TABLE 35.10 Effect of Processing on ORAC Values.

	ORAC values (mmol)
Fresh (82.3% moisture)	52.9
IQF (84.5–87.8% moisture)	31.2–39.3
Puree (87.2% moisture)	42.0
Canned fruit (74.6% moisture)	18.7
Pie filling (63.4% moisture)	6.49
Jam (72.7% moisture)	10.6
Juice concentrate (50.5% moisture)	29.4
Dried (33.8% moisture)	25.5
Dried (16.8% moisture)	15.1
Sugar infused-dried (28.2% moisture)	28.2
Dried fruit for cereal (7.8% moisture)	2.97

contained 1% chlorogenic acid and 18% anthocyanins. The anthocyanin pigment malvidin was more stable than delphinidin, and the losses in anthocyanins and phenolics during milling and depectinization were believed to be due to native polyphenol oxidase in blueberries. Lee and others (2002) showed that blanching blueberries (95°C for 2 min) or treatment with 50 ppm potassium metabisulfite before juice processing minimized anthocyanin loss in blueberry juice.

Kalt and others (2000) reported the effect of various processing steps on ORAC (mmol Trolox equivalent/100 g dry weight) values of wild blueberries as in Table 35.10. Dried fruit for cereal had the lowest ORAC value, followed by pie filling and jam.

Chemopreventive and Anti-Adhesion Properties of Wild Blueberry. Wild blueberry fractions containing 4–8-linked oligomeric proanthocyanidins with average degrees of polymerization (DPn) of 3.25 and 5.65 were shown to inhibit adhesion of *E. coli*, which is responsible for urinary tract infections. The fraction with a DPn of 5.65 showed antiproliferation activity against human prostate and mouse liver cancer cell lines (Schmidt and others 2004).

Kraft and others (2005) showed that a wild blueberry extract containing phytosterols was active against initiation (quinone reductase assay) of carcinogenesis; fractions containing flavan-3-ols and anthocyanins, phenolic acids, flavan-3-ols, and some proanthocyanidin dimers demonstrated activity against the promotion stage (cyclooxygenase and ornithine decarboxylase assays), and a proanthocyanidin-rich fraction had antiproliferation (progression stage) activity.

Schmidt and Others (2005) showed that in both cultivated and wild blueberries, fresh and IQF blueberries had more total phenolics, antioxidant activities, and antiproliferative activity than processed products. Further, except for freeze-dried wild blueberry (freeze-dried cultivated was not analyzed), all other heat-treated blueberries lacked or had

TABLE 35.11 Nutrients in Blueberries.

Nutrients/100 g	Blueberries, Raw[a]	Canned Blueberry in Syrup[a]	Infused-Dried Cultivated Blueberries[b]	Infused-Dried Wild Blueberries[b]	Infused-Dried Organic Wild Blueberries[b]
Calories (kcal)	57.0	88.0	290	305	280.0
Calories from fat (kcal)	3.0	3.0	20.0	19.0	11.0
Total fat (g)	0.33	0.33	2.19	2.06	1.17
Trans fat (g)	<0.10	<0.10	<0.10	<0.10	<0.10
Cholesterol (mg)	0.00	0.0	0.0	0.0	0.0
Sodium (mg)	1.0	3.0	18.0	15.0	22.0
Potassium (mg)	77.0	40.0	252.0	166.0	144.0
Total carbohydrate (g)	14.49	22.06	77.9	80.3	78.6
Total fiber (g)	2.4	1.6	16.6	15.4	15.1
Total sugar (g)	9.96	20.46	61.2	64.9	60.5
Protein (g)	0.74	0.65	2.03	2.43	0.84
Calcium (mg)	6.0	5.0	255.0	380.0	49.0
Vitamin C (mg)	9.7	1.1	<0.10	76.0	0.10
Vitamin A (IU)	54.0	36.0	14.0	33.0	4.0

NA: Not available.

[a]http://www.nal.usda.gov/fnic/foodcomposition/cgi-bin/list_nut_edit.pl

[b]Courtesy of Graceland Fruit Inc, Frankfort, MI, USA (www.gracelandfruit.com)

diminished antiproliferative activity. However, appreciable antioxidant capacity and phenolic compounds were retained even in intensively processed products like jam and pie filling.

35.2.3.4 Nutritional Quality. Table 35.11 shows nutritional values of blueberry and its products. Besides the bioactive phytochemicals for which the blueberries have become best known, these berries are also a good source of fiber, natural sugars, vitamins, and minerals.

35.2.4 Blueberry Products

Similar to strawberries, blueberries are processed into traditional products such as jam and jelly, juice, puree, canned, and dried. Premium value-added products such as infused-frozen, infused-dried, freeze- and vacuum-dried blueberries have been developed for specific uses. An overview of blueberry products is described in the following.

35.2.4.1 IQF Blueberries. Following cleaning and sanitation (with 20–35 ppm chlorine water spray) steps, the berries are conveyed through a freezing tunnel (with high-velocity cold air at -40°C) for about 10 min. The IQF berries are destemmed, color sorted, visually inspected, metal detected, packed, and stored frozen at $<-18^{\circ}$C. The berries, that do not meet IQF quality grades, are culled and used for processing into juice, puree, and so on.

35.2.4.2 Blueberry Puree. Similar steps as described for strawberry puree are followed for making blueberry puree and puree concentrates. Berries are cut/crushed, passed through a pulper/finisher, pasteurized or concentrated, and filled into containers.

35.2.4.3 Blueberry Juice and Concentrate. Blueberry juice is generally marketed as a blend with other juices. The basic juice-making steps are similar to strawberries. Single-strength blueberry juice is about 8–12° Brix. Frozen blueberry juice concentrates of 45–65°Brix are made by concentrating single-strength blueberry juice and freezing to less than -18°C. The blueberry essence is captured from the concentration process and blended back into the concentrate and also sold separately.

35.2.4.4 Infused and Stabilized Frozen Blueberries. These products are specifically made for use in bakery and dairy products. They are infused with sugar, corn syrup, or fruit juices to about 25–45° Brix and heat processed. The processing is similar to infused and stabilized strawberries. The berries thus processed are soft and do not undergo syneresis (release water) like IQF berries when thawed.

35.2.4.5 Infused-Dried Blueberries. Infused-dried blueberries have a moisture content of about 10–15% and a water activity of 0.40–0.60. The berries thus dried have a fresh-like texture, color, and flavor, and are shelf stable. They rehydrate well and can be used as ingredients in many food applications. The process is similar to infused dried strawberries and apples.

35.2.4.6 Freeze-Dried and Vacuum Dried Blueberries. As indicated for strawberries, freeze-drying is a long (almost 24 h) process, and the cost of the freeze-dried

products is about 3–5 times higher than products dried by other methods. However, as the freeze-dried blueberries are light, crispy, porous, and hold shape better, they are desirable in ready-to-eat cereals. A published patent application (Bauman and others 2006) describes the process for vacuum puffed and expanded fruits including blueberries. In this process, the berries are lightly infused and then processed under specific vacuum and heating conditions to expand/puff and dry the blueberries. The product is claimed to have crispy and light texture, low water activity, and low buoyant density.

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36

Major Tropical Fruits and Products: Banana, Mango, and Pineapple

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36.1 Introduction 816
36.2 Banana 817
36.2.1 Introduction 817
36.2.1.1 Historical Brief 817
36.2.1.2 Classification 817
36.2.1.3 Cultivation 817
36.2.2 Supply and Demand 818
36.2.2.1 Supply 818
36.2.2.2 Demand 819
36.2.3 Harvest, Postharvest Handling, Ripening, and Storage 819
36.2.4 Physico-Chemical, Nutritional, and Phytonutrient Quality 820
36.2.4.1 Phenolic Compounds and Antioxidant Capacity 820
36.2.5 Banana Products and Processing 821
36.2.5.1 Banana Puree 821
36.2.5.2 Banana Chips 825
36.2.5.3 Other Processed Banana Products 826
36.3 Mango 826
36.3.1 Introduction 826
36.3.1.1 Historical Brief 826
36.3.1.2 Classification 826
36.3.1.3 Cultivation 826

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

36.3.2 Harvest, Postharvest, Ripening, and Storage 827
36.3.3 Supply and Demand 827
36.3.3.1 Supply 827
36.3.3.2 Demand 828
36.3.4 Physico-Chemical, Nutritional, and Phytonutrient Quality 828
36.3.4.1 Physico-Chemical 828
36.3.4.2 Mango Flavor 828
36.3.4.3 Nutritional Content 828
36.3.4.4 Phenolic Content 829
36.3.5 Mango Processing 829
36.3.5.1 Mango Puree 829
36.3.5.2 Mango Juice Concentrate 832
36.3.5.3 Mango Juice 832
36.3.5.4 Mango Slices and/or Scoops 832
36.3.5.5 Mango Leather and Mango Bar 832
36.3.5.6 Other Mango Products 833
36.4 Pineapple 833
36.4.1 Introduction 833
36.4.1.1 Historical Brief 833
36.4.1.2 Classification 833
36.4.1.3 Cultivation 833
36.4.2 Harvest, Postharvest, and Storage 833
36.4.3 Supply and Demand 834
36.4.3.1 Supply 834
36.4.3.2 Demand 836
36.4.4 Physico-Chemical, Nutritional, and Phytochemical Quality 836
36.4.4.1 Physico-Chemical Quality 836
36.4.4.2 Flavor Components in Pineapple 836
36.4.4.3 Nutritional Composition 836
36.4.4.4 Phenolic Content and Antioxidant Compounds 836
36.4.5 Pineapple Processing 837
36.4.5.1 Selected Pineapple Products 837
References 842
Websites 845

36.1 INTRODUCTION

Tropical fruits, by definition, are products of warm climates and equatorial regions of the world. Major commercial tropical fruits such as banana, mango, and pineapple are sources of important nutrients in our diet. For example, banana is an excellent source of potassium and slow digesting starch, mango is a rich source of vitamin A, and pineapple is one of the lowest caloric fruits and a source of vitamin C. Recognizing the diet–health relationship, the USDA's new "MyPyramid" and the 2005 U.S. Dietary Guidelines emphasized fruit and vegetable consumption. Tropical fruits, as a part of the fruit basket with special flavor and taste appeal, can help in meeting our dietary goals for fruits and vegetables. Recent growth of tropical fruits in the United States can be attributed to an ethnically (particularly, Hispanics and Asians) diverse population. This market is also creating new tropical-fruit-based products (mango salsa, tropical fruit beverage blends, and so on). Further, a continuing influence of ethnic cuisine, for example, Nuevo Latino cuisine, in the food service and food industry provides a venue for the utilization of tropical fruits. This chapter provides an overview of the horticulture, supply, demand, physico-chemical, nutritional, and processing aspects of three major commercial tropical fruits: banana, mango, and pineapple.

36.2 BANANA

36.2.1 Introduction

36.2.1.1 Historical Brief. The origin of bananas (*Musa* sp.; banana from Arabic for "finger") is located in Southeast Asia. The first Europeans to come across bananas were the army of Alexander the Great during their invasion of India in 327 B.C. However, Portuguese and Spanish explorers brought bananas to the New World. The international trade in bananas started towards the end of the nineteenth century as a result of developments in railroads and refrigerated maritime transport. The early processed banana products imported into the United States came from Brazil, Honduras, and Mexico, and included dried and evaporated bananas (Von Loesecke 1949). At present, major exporters of processed banana products to the United States are Latin American and Asian countries.

36.2.1.2 Classification. Bananas are members of the genus *Musa* (family *Musaceae*) and include dessert bananas (fresh eating quality, soft and sweet: *Musa acuminata*) and plantains (starchy, less sweet, cooking or processing bananas: *Musa balbisiana*). Among important banana cultivars for fresh consumption are "dwarf" and "giant" Cavendish, and Gros Michel. The subgroup of "giant" Cavendish, *Grand Nain* (has thick skin and can withstand bruising) and is the most common cultivar imported into the United States, where consumers prefer bananas with unblemished skins. Subtropical Baby Bananas (*Lady Finger*, *Oritos*, *Manzanos*, or *Apple*) have thin skin with a yellow-pinkish cast and are very sweet. Other sweeter and thin-skinned cultivars (*Senorita*, *Lacatan*, and *Sucrier*) are small and easily brown.

36.2.1.3 Cultivation. Bananas are herbaceous giant perennial plants grown from corms, rhizomes, or suckers. They can normally be found in the field as a cluster of plants with the most mature one known as the leader surrounded by a couple of growing suckers (Fig. 36.1). Modified leaves compactly cluster around each other as the plant height increases, each of which ultimately unfurls to the familiar banana "leaves". At the center of these modified leaves will emerge a modified growing point in the shape of a "heart", which houses the plant's inflorescence. The inflorescence is covered by a flap structure arranged in layers. The floral cluster in between two flaps consisting of a row of 15 to 30 individual flowers will ultimately develop into a "hand" of the banana bunch, with each "finger" of the "hand" representing the individual flowers. As each hand increase in size, it forces the flap to open up wider. The next lower flap will follow the same series of steps. When a banana bunch is fully grown it will be composed of 7–10 hands spiraling around the fruit axis. The leader will eventually die off and the next mature sucker will take its place.

The banana plant thrives in tropical climates (temperatures around 80°F, annual rainfall of 79–98 in., moist soil with good drainage), and generally grown within 30° North and South latitude. Growth ceases when temperatures drop below 56°F, causing chilling injury to the banana fruit. Minimal hurricane winds can uproot plants bearing heavy fruit stalks, and bring damage to an entire plantation.

Tissue cultured plantlets are now used to start new plantations. Micropropagated banana plants provide pest-free propagation materials for starting new plantings, but require more investment, including a nursery.

Figure 36.1 *Growth of Bananas A-corm; B-Rhizome; C-Tightly-packed unopened leaves; D-Sucker (single); E-Stool (group of suckers); F-Inflorescence (unopened); G-Bunch growing with the inflorescence; H-Bunch of "hands" and "fingers".*

36.2.2 Supply and Demand

36.2.2.1 Supply. About 20% of bananas are exported from the developing to developed countries, an example of unidirectional, South–North trade. The world production of bananas in 2005 was 72,464,562 metric tons, grown over 4,349,485 hectares (Table 36.1). Asia and Latin America contributed about 64% and 33%, respectively, of world banana production in 2005. India ranks number one in banana production. However, its internal demand and consumption is relatively high and, as a result, not much enters into the export market. The Philippines, which ranks fifth in world production of fresh bananas, is a leading exporter of banana chips to the U.S. market. Ecuador is the top exporter of banana flakes.

TABLE 36.1 Leading Countries and World Aggregate in Banana Production (2001–2005).

Country	Production (Mt)		Area Harvested (Ha)	
	Average (2001–2005)	2005	Average (2001–2005)	2005
1. India	16,298,000	16,820,000	638,000	680,000
2. Brazil	6,542,080	6,702,760	501,518	494,462
3. China	6,004,626	6,390,000	263,974	274,200
4. Ecuador	5,880,729	5,877,830	215,760	209,027
5. Philippines	5,428,529	5,800,000	407,946	430,000
6. Indonesia	4,427,973	4,503,467	290,740	315,000
7. Costa Rica	2,129,600	2,220,000	43,960	45,700
8. Mexico	1,998,727	2,026,610	70,381	72,645
9. Thailand	1,890,000	2,000,000	145,200	153,000
10. Colombia	1,547,822	1,600,000	57,569	62,000
World	70,133,020	72,464,562	4,322,081	4,349,485

Source: FAO (2006).

Plantain yields are lower than the dessert banana. They serve as a major staple food in many countries. In 2005 world plantain production (33,407,921 Mt) was dominated by the African countries Uganda (30%), Rwanda (8%), Ghana (7%), Nigeria (6%), Cameroon (4%), Cote d'Ivoire (4%), and Congo (1%); Latin American countries were led by Colombia (10%).

36.2.2.2 Demand. Banana is perhaps the most consumed fruit in the world. One reason is that it is relatively inexpensive, and it is also easily digestible by the very young and old alike. Bananas are not domestically grown in the United States, yet it ranks top among fresh fruit consumption, with average per capita consumption of over 28 lb. Most U.S. imports of bananas are from Costa Rica, Honduras, and Guatemala. Bananas are also liked in Europe and the per capita consumption in Sweden, for example, is 35 lb. The EU considers banana a fair trade commodity from certain Pacific, Asian, and African countries.

36.2.3 Harvest, Postharvest Handling, Ripening, and Storage

The interval between inflorescence/flowering and harvest of bananas is about 8–10 months, and there is year-round production. Bananas are harvested from the plants when light green in color and about 75% mature. Entire bunches (Fig. 36.1) are cut by hand from pseudostems. For local consumption, the hands are left on the stalks and sold to vendors who cut hands/fingers according to customer specifications. In large-scale operations, the banana bunches are carried to a nearby tramline or cableway for transport to the packinghouse. Banana bunches hanging on tramways are pulled by tractors to minimize handling and bruising of the fruits. Bananas for export are treated by floating in water or dilute sodium hypochlorite solution to remove latex, which causes black peel staining. Hands are cut into units of 4–10 fingers, graded for both length and width, and carefully placed in poly-lined 40 lb boxes for export.

Fruits are shipped when they are green, and ripened by exposure to ethylene ($\sim$1000 ppm for $\sim$24 h) in sealed "banana ripening rooms". Palmer (1971) discussed in detail respiratory and compositional changes during ripening of bananas. Bananas

should be protected from light after harvesting to delay softening and onset of ripening. Bananas are susceptible to chilling injury; storage temperature should not be lower than 55°F. Gibberellins, reduced temperatures, and modified atmospheres have all been reported to delay the onset of banana ripening (Kapoor and Turner 1976; Taylor 2001). A seven-point color index scale (1 = all green; 2 = with trace of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = green tips and necks; 6 = all yellow; 7 = yellow with brown flecks) describes various stages in ripening of bananas (Nakasone and Paull 1998). A computer vision system using Hunter color L^*, a^*, b^* bands and brown area percentage has been used to predict the seven ripening stages of banana (Mendoza and Aguilera 2004).

36.2.4 Physico-Chemical, Nutritional, and Phytonutrient Quality

The edible portion of banana contains about 5% starch and 12% sugars. It is one of the few low-acid fruits (pH of about 5.0). Ripened banana has a pleasant flavor and creamy yellow color. Isoamyl acetate is responsible for overripe sweet banana flavor. Table 36.2 summarizes the nutritional composition of banana and its products. Bananas contain more digestible carbohydrates and a relatively higher starch content than any other fruit. One fresh banana has about 15% vitamin C, 20% vitamin B_6, 11% potassium, and 16% dietary fiber needed as per the Recommended Daily Allowance. The American Heart Association has certified Chiquita® bananas as meeting their food criteria for saturated fat and cholesterol for healthy people over two years of age.

36.2.4.1 Phenolic Compounds and Antioxidant Capacity. A strong water-soluble antioxidant, dopamine, has been identified in the popular commercial banana *Musa cavendishii*. The peel and pulp of ripened banana were shown to contain 80–560 mg and 2.5–10 mg dopamine respectively, per 100 g (Kanazawa and Sakakibara 2000). Dopamine (through its condensation product salsolinol), however, is implicated in

TABLE 36.2 Composition of Banana Fruit and Banana Products.

	Nutrients/100 g		
	Fruit[a]	Powder[b]	Puree[c]
Calories (kcal)	89	346	95
Total fat (g)	0.33	1.81	0.80
Cholesterol (mg)	0	0	0
Sodium (mg)	1.0	3.00	8.30
Potassium (mg)	358.0	1491	298.0
Total carbohydrate (g)	22.84	88.28	21.3
Total dietary fiber (g)	2.6	9.9	2.00
Total sugar (g)	12.23	47.30	18.60
Calcium (mg)	5.0	22	4.40
Iron (mg)	0.26	1.15	0.32
Vitamin C (mg)	8.7	7.0	5.66
Vitamin A (IU)	64	248	93.0
Protein (g)	1.09	3.89	0.65

[a]USDA National Nutrient Database: http://www.ars.usda.gov/nutrientdata
[b]Adapted from Von Loesecke (1949).
[c]Acidified Banana Puree, iTi tropicals: http://www.bananapuree.com/specs2.php

TABLE 36.3 Total Phenolics and Antioxidant Capacity of Banana, Mango, and Pineapple.

Fruit	Moisture (%)	TP[a] (mg GAE/g)	TAC[b] (μmol TE/g)	Serving Size (g)	TAC/Serving (μmol TE)
Banana	73.5	2.31 ± 0.60	8.79	118.0	1037
Mango	81.7	2.66	10.02	165.0	1653
Pineapple	86.8	1.74 ± 0.52	7.93	155.0	1229

Source: Wu and others 2004.

[a]TP = Total phenolics determined as gallic acid equivalent.
[b]TAC = Total antioxidant (lipophilic and hydrophilic oxygen radical absorbance capacity) as Trolox equivalent.

the appearance of black spots in overripe bananas (Palmer 1971; Riggin and others 1976). Table 36.3 shows antioxidant capacity and total phenolic content of banana fruit. Because we consume banana so extensively, it is an excellent contributor of natural antioxidants to our diet.

36.2.5 Banana Products and Processing

The "cooking" banana or plantains are preferred for processing (especially for making banana chips, and so on) over the "dessert" or table bananas due to their higher starch content. They are also less susceptible to enzymatic browning due to polyphenol oxidase (PPO), and to discoloration as a result of the reaction between metal ions and the vascular bundles loosely attached to the banana skin ("peel rag") (Occeña-Po 2006). Figure 36.2 presents the utilization and processing of banana, and the use of some commercially processed banana products is shown in Table 36.4.

36.2.5.1 Banana Puree. Banana puree (with or without seeds) is the most important and the highest volume processed banana product (Sole 2005). It is available either in canned or frozen forms. It is incorporated into the preparation of many retail products (Figs 36.2 and 36.3, Table 36.4), even catsup.

Figure 36.3 summarizes the processing steps involved in the manufacture of aseptic banana puree (and banana essence). Both cold and evaporator-recovered essence can be

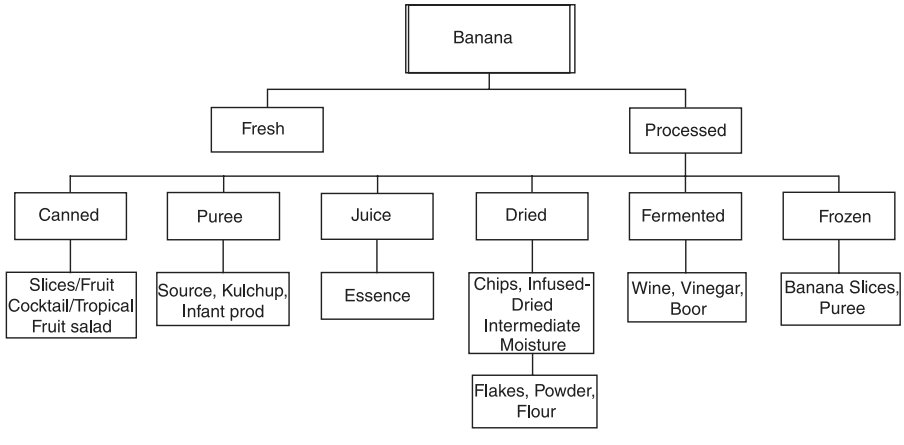


Figure 36.2 *Banana utilization and products.*

TABLE 36.4 Commercially Processed Banana Products.

Product	Characteristics	Packaging	Shelf-Life	Applications
Sliced and whole peeled[b,g]	Ripened, peeled and frozen; for use when piece identity is essential	33 lb carton	Frozen (0°F)	In lieu of fresh bananas; beverages; dairy products; bakery products
Sliced[b]	• Packed in high-fructose corn syrup, color stable, sweetened	Resealable package	Shelf-stable	Bakery products; dairy products; food service and institutional products; used when fresh banana appearance/identity is desired
	• Packed in light syrup; processed in acidified light sugar syrup	No. 10 cans	No refrigeration required until opened	
Puree[a,c,f]	Brix: 21–25°; 22–26°[f] TSS: 23–28%[f] pH: 4.6–5.2[f] Acidity: 0.35–0.7% (as citric acid)[f] Viscosity: 3–9 cm/s at 68°F	1 × 5 gal bag-in-box; 1 × 55 gal bag-in-drum; 1 × 220 gal bag-in-tote; 275 gal tote	12 months (59–86°F) in sealed containers	Baby food; fruit preparations; beverages; dairy products; bakery products; frozen desserts and soft food diets; catsup; sauce
Acidified puree[a,c,f]	pH: 4.2–4.5[f] (citric/ascorbic acid)	6 gal bag-in-box and 1 L Tetra Pack[f]	Room temperature, 74°F	
Concentrated juice[e]	Brix: 72 ± 1° pH: 4.0–4.3 TA: 0.95–1.45%	300 kg drums; 25 kg plastic pails	Frozen (0°F)	Ingredient
IQF	3/8 in.	33 lb carton	Frozen (0°F)	Fruit preparations; frozen desserts; bakery products

Flakes[a]	Moisture <2.5%	40 lb bag-in-box 44.1 lb bag-in-box Heat-sealed polyethylene bag	18 months (69.8°F)	Dry fruit preparations; substitute for fresh mashed bananas (1 : 3 reconstitution); fruit preparations; beverages; bakery products; dairy products; cereals[c]
Powder[a,d]	Moisture ≤3.0%	30.5 lb bag-in-box	18 months (69.8°F)	Dry fruit preparations; beverages; bakery products; substitute for fresh mashed bananas (1 : 3 reconstitution); fruit preparations; dairy products; baby food
Diced dried pieces[b]	$A_w \leq 0.55$; varied sizes and shapes		Shelf-stable	Cereals; bakery products; dairy products; snacks
Puree extract[b]	Natural banana aroma and bouquet of ripe bananas; heat stable; alcohol-based		Shelf-stable	Beverages; dairy products; bakery products
Essence[a]	Dosage 1% by weight and adjust up or down as desired	20.8 lb plastic jugs; 45 lb per carton; 484 lb plastic drum; 507 lb	12 months (44.6°F) absence of air and light	Applications in water-based system to impart banana flavor, e.g., refrigerated beverages; dairy products; combination with puree to enhance aroma of product
Tostones[b]	Ripened, sliced, fried in soybean oil, cooled		Frozen	Breakfast (mashed and egg scrambles); appetizers; lunch; dessert

Source: [a]Trobana® Banana Products, Confoco, www.confoco.com/confoco/ingles/productos.htm.; [b]Chiquita Banana Products, www.chiquita.com/doingbusiness/fiproduct.asp; [c]Also available as nonacidified form; [d]Also available in organic form; [e]Florida Products, adapted from Sole (2005); [f]iTi Tropicals, www.bananapuree.com; [g]iTi Tropicals, http://www.ititropicals.com/ProductList.pdf.

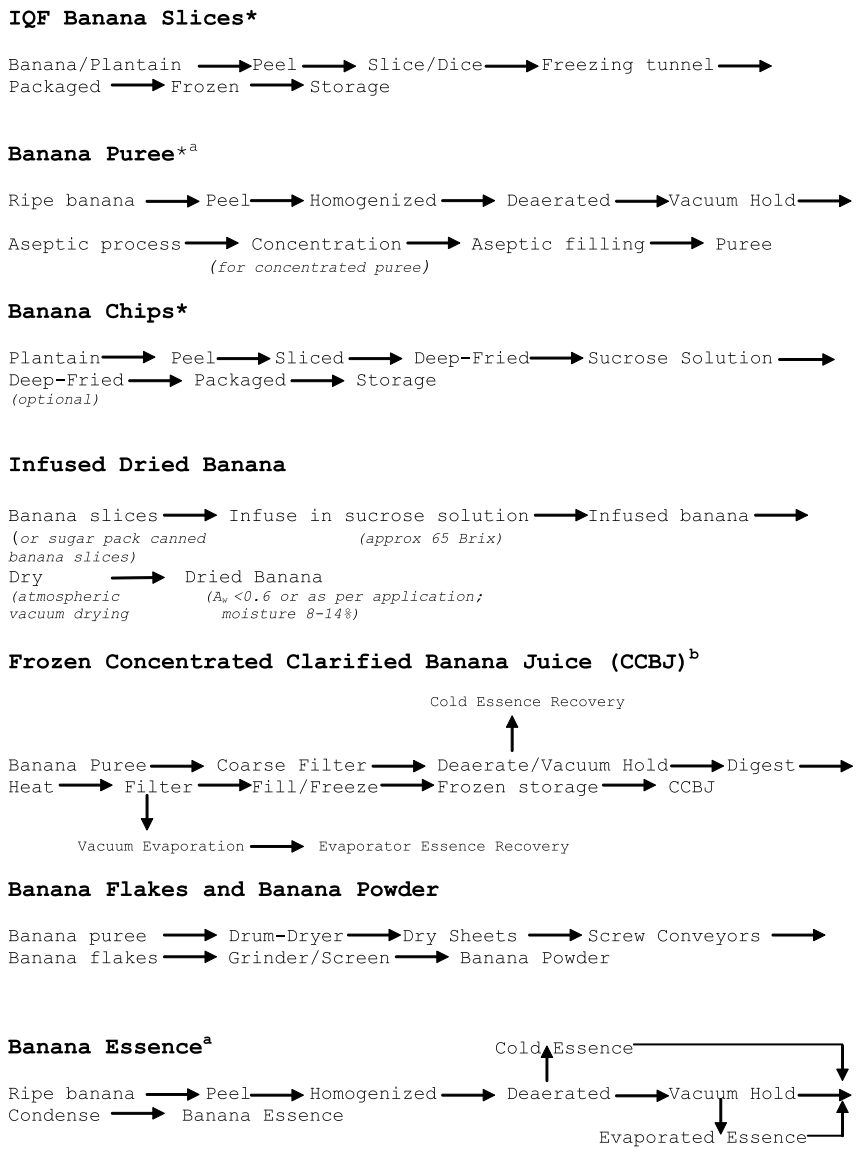


Figure 36.3 *Processing of selected banana products.*

added back to the banana puree to enhance flavor profile. Banana puree is mixed with other fruit purees (e.g., in formulation of baby foods) to provide various fruit flavor characteristics.

An acidification (use of ascorbic acid and citric acid to prevent browning) step is often followed in making banana puree. Johnson and Harter (1981) used an extrusion process: heating up to 249.8°F and cooling to 35–37°F, filling, and blast freezing (−4°F) to produce banana puree. Others (Palou and others 1999; Premakumar and Khurdiya 2002) have investigated the use of high hydrostatic pressure, microwave (3 min), and

water bath (212°F; 8 min) blanching of bananas to retard enzymatic browning. Banana puree can also be prepared without freezing or sterilization, using citric acid (to lower the pH to 4.1–4.2) and potassium sorbate (200–250 ppm) (Downing 1996).

Banana catsup (ketchup) is a popular substitute for tomato catsup in the Philippines, and is usually prepared from fresh banana pulp or puree mixed with vinegar, salt, sugar, onion powder, and spices. The product is mainly exported to the United States, the UK, mostly for people of Philippine descent (Sole 2005; Occeña-Po 2006).

36.2.5.2 Banana Chips. In the Philippines, Green *Saba* (plantain), a cooking banana, is utilized for making banana chips. Figure 36.3 summarizes the main steps involved. After osmotic dehydration (soaking in sugar solution) and deep-frying (~375°F for 1 min) in coconut or other oils, banana chips can be air-dried at room temperature (~81°F), or cabinet dried (~140°F) to allow for moisture equilibration. For making natural banana chips, the osmotic dehydration step is skipped. The light-colored banana chips are exported in bulk and repacked in moisture-resistant laminated plastic pouches (Fig. 36.4*a*). They can be used as snacks or incorporated in fruit trail mixes or ready-to-eat breakfast cereals (Sole 2005; Occeña-Po 2006).

To improve the quality and color of banana chips, studies have looked at

1. Browning inhibitors, osmotic dehydration, microwave, sulfiting agents, water- or steam-blanching prior to deep-fat frying, and freeze-drying (Woodroof 1975; Krokida and others 2000; Waliszewski and others 2000; Krokida and others 2001);
2. Combination of air and vacuum-microwave drying (Mui and others 2002); and
3. Effects of packaging during storage on the color of banana chips (Ammawath and others 2002).

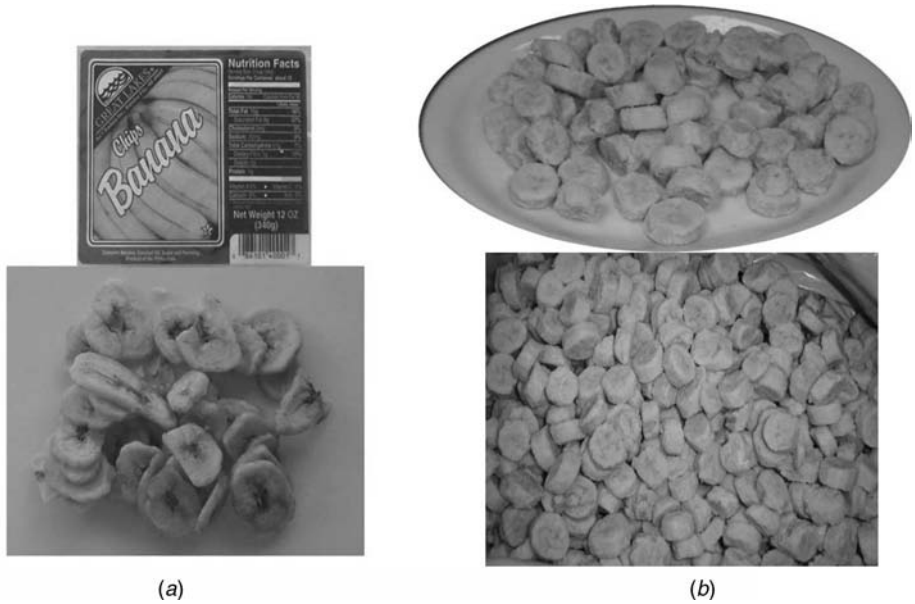


Figure 36.4 *(a) Dried banana chips and (b) Individually Quick Frozen (IQF) baby banana samples.*

36.2.5.3 Other Processed Banana Products. Figure 36.3 and Table 36.4 provide the processing and product descriptions, respectively, of commercial banana products. Figure 36.4*b* is a photograph of Individually Quick Frozen (IQF) plantain banana slices, prepared as summarized in Figure 36.3. IQF bananas are utilized in many product applications.

36.3 MANGO

36.3.1 Introduction

36.3.1.1 Historical Brief. The cultivated mango (*Mangifera indica* L.) originated in southeastern Asia, and was brought to England during the colonization of India in the 1800s. Mangoes were introduced in Brazil and the West Indies during the 1700s, and to Florida in the late 1800s. Portuguese and Spanish traders brought the mangoes to East Africa, the Philippines, and western Mexico.

36.3.1.2 Classification. Mangoes are large fleshy drupe fruits in which the hard stone (endocarp) containing a single seed is covered with an edible juicy pulp. There are two classes of mango:

1. The "Indochinese" cultivars characterized by kidney-shaped, elongated and flattened fruit with light green or yellow skin (Fig. 36.5*a*); and
2. The "Indian" cultivars described as rounded and plump, having a bright red blush to the skin (Fig. 36.5*b*). Indian cultivars are monoembryonic (e.g., *Alphonso*, *Malda*, *Totapuri*, *Kent*, *Tommy Atkins*), so they are mostly grafted on seedling rootstocks and bear fruit much earlier than do seedling trees, which is the case for polyembryonic Indochinese mangoes (e.g., *Carabao*, *Cambodian*, *Kaewsaard*) (Rieger 2005). Knight (1997) has provided a review of the important mango cultivars in the world.

36.3.1.3 Cultivation. Mangoes grow best in seasonally wet/dry climates of the lowland tropics, or frost-free subtropical areas. Mangoes can grow in many soil types

(*a*) (*b*)

Figure 36.5 *(a) Typical appearance of the Indian cultivar and (b) typical appearance of the Indochina cultivar.*

provided they are adequately drained and mildly acidic, although they are grown on limestone gravel in Florida. Commercial growers maintain the mango trees in cultivated orchards. However, in many mango-producing countries, it is not uncommon to find them as backyard trees or along the roadside. In India, the mango tree is considered auspicious and mango leaves are used during worship and other important ceremonies.

36.3.2 Harvest, Postharvest, Ripening, and Storage

Mango is a climacteric fruit, which is best harvested in a mature but unripe stage about $2\frac{1}{2}$ months to $4\frac{1}{2}$ months from blooming. Fruits are essentially hand-picked and pickers use poles to reach fruits high up in the tree. Other devices such as ladders and hydraulic lifts can also be used. Mangoes are usually transported in the firm, green preclimacteric stage. Following harvesting, mangoes should be dipped in hot water (122–127°F; 15 min) as a postharvest control of mango anthracnose – a destructive pest. Additionally, this postharvest dip helps in the removal of the latex sap that can cause darkening.

Among other factors, the ripening of mango depends on variety, degree of maturity at harvest, storage temperature, and humidity. For the purpose of ripening, about one week of storage at 72–75°F (relative humidity of about 85–90%) enables development of mango flavor. Ethylene can also be used to effect ripening. The shelf-life of mango will vary depending on the initial quality of the fruit and storage temperatures (2–3 weeks storage life at 41–50°F is not uncommon). However, mangoes are susceptible to chilling injury (pulp having brown/dark spots, and discoloration of skins) when stored at temperatures near freezing.

36.3.3 Supply and Demand

36.3.3.1 Supply. The export market has been led by India (Table 36.5), and so by the Indian cultivars (several are of Florida origin), initially by *Haden*, but now by other cultivars similar in appearance, including *Tommy Atkins*, *Keitt*, *Kent*, *Palmer*, and *Irwin*. The United States imports most of its mangoes from Mexico. A total of 27,966,749 metric tons of mangoes were produced in 2005, up by only 4% over the average production of the last

TABLE 36.5 Leading Countries and World Aggregate in Mango Production (2001–2005).

	Production (Mt)		Area Harvested (Ha)	
Country	Average (2001–2005)	2005	Average (2001–2005)	2005
1. India	10,616,000	10,800,000	1,580,000	1,600,000
2. China	3,522,353	3,673,000	408,716	433,600
3. Thailand	1,720,000	1,800,000	273,000	285,000
4. Mexico	1,521,928	1,503,010	169,143	173,837
5. Indonesia	1,353,709	1,478,204	214,687	273,440
6. Pakistan	1,158,281	1,673,900	110,673	151,500
7. Philippines	952,290	950,000	152,315	160,000
8. Brazil	849,935	850,000	67,608	68,000
9. Nigeria	730,000	730,000	125,000	125,000
10. Vietnam	269,140	320,000	55,660	53,000
World	26,727,864	27,966,749	3,686,316	3,870,200

Source: FAO (2006).

five years. These figures may not actually account for all mangoes produced, especially those in family backyards and in small farms.

36.3.3.2 Demand. Fresh mangoes used to be sold only in ethnic stores/markets in the United States, but some varieties can now be easily spotted in the produce section of many supermarkets. Imported mangoes are mostly processed into puree and juice. The per capita consumption of processed mango products in the United States is higher than the consumption of fresh mangoes.

36.3.4 Physico-Chemical, Nutritional, and Phytonutrient Quality

36.3.4.1 Physico-Chemical. The chemical composition of ripe mangoes varies with the cultivar. Moisture content ranges from 72% to 86%; soluble solids from 14 to 23°Brix; pH from 3.8 to 5.6, and acidity (as citric acid) from 0.11 to 0.48%. Major organic acids include citric, tartaric, oxalic, malic, and glycolic. Ripe mangoes have a relatively higher sucrose content compared to reducing sugars (3–10%), and total sugars can ranges from 9% to 21% (Hulme 1971; Nanjundaswamy 1997).

36.3.4.2 Mango Flavor. The major volatiles found in mango are monoterpene hydrocarbons. In canned mango puree, the volatiles identified included butyrolactone, gamma-octalactone, furfural, and 5-methyl furfural methoxy furanone (Hunter and others 1974). A commercial water-soluble distillate (Treattarome 9830) derived from ripe mango contains high levels of fruity esters, lactones, spicy terpenes, and sugary/caramel-like furanones.

36.3.4.3 Nutritional Content. The nutritive value of mango products varies with cultivar, cultural and climatic conditions, ripeness, postharvest storage, and processing. Table 36.6 shows that mangoes are excellent source of vitamin A. It is also a source of

TABLE 36.6 Nutrients in Mango and Mango Products.

Nutrients/100 g	Fruit[a]	Infused-Dried Fruit[b]	Slices[c]
Calories (kcal)	65.0	331	70
Total fat (g)	0.27	0.78	0
Cholesterol (mg)	0.0	<0.1	0
Sodium (mg)	2.0	24	15
Potassium (mg)	156.0	182	—
Total carbohydrate (g)	17.0	83.1	19
Total fiber (g)	1.8	6.1	<1
Total sugar (g)	14.80	75.6	17
Protein (g)	0.51	0.67	0
Calcium (mg)	10.0	28.0	0
Iron (mg)	0.13	0.83	0
Vitamin C (mg)	27.7	262.0	100%
Vitamin A (IU)	765.0	1554	15%

Sources: [a]http://www.ars.usda.gov/ba/bhnrc/ndl; [b]Data of commercial products: Infused with sugar prior to drying, contains added ascorbic acid and high oleic sunflower oil. Courtesy of Graceland Fruits Inc., Frankfort, MI, USA; [c]http://www.delmonte.com/Products/FruitItem.asp?id=46; SunFresh® Mango (124 g serving size).

vitamin C. The vitamin B complex content is relatively low, except for niacin (Wu and others 1993).

Little loss of carotenoid pigment occurs during freezing of mango slices, although there is some decline with storage. The loss of β-carotene during candied mango processing has been shown to be 17–18%, which increases to 30–40% during storage (Chavasit and others 2002). Ascorbic acid retention in mangoes frozen by liquid nitrogen is 90% compared to 72% by slow tray freezing (Pruthi 1999).

36.3.4.4 Phenolic Content. Phenolic content (31–75 mg tannic acid equivalent/100 g flesh) of ripe mango is influenced by variety and size (Hulme 1971; Narain and others 1998). Table 36.3 gives total phenolic content and total antioxidant capacity of the mango fruit.

36.3.5 Mango Processing

Figure 36.6 shows utilization of mango and processed mango products. With the exception of mango chutneys and pickled mango products, which utilize immature or green mangoes, fully ripened mangoes with well-developed flavor, color, and texture are preferred for processing into mango products. Some mango cultivars, which are too fibrous or soft for fresh consumption, can be used for juice making. This section describes major processed mango products.

36.3.5.1 Mango Puree. Mango puree is processed either as a canned or frozen product. It serves as the starting raw material for mango beverages, jams, and dehydrated products, and is also incorporated into infant foods, bakery, dairy, and probiotic products. Various mango cultivars, which differ in their sensory attributes, can be blended and made into mango puree. Whole (for cultivars with thin and yellow color peels) or peeled mangoes can be utilized. Figure 36.7 shows the basic steps involved in manufacturing canned and frozen mango puree, and Table 36.7 provides Brix ranges and packaging

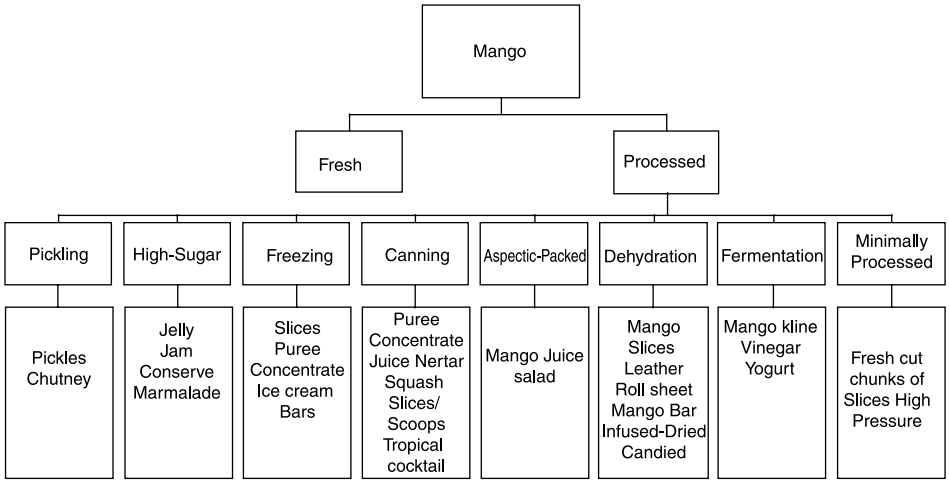


Figure 36.6 *Mango utilization and mango products.*

Mango Puree

Canned

Ripe Mangoes → Wash → Peel → Slice → Pulper → Centrifugation →
(30 mesh sieve)
Finishing → Heating → Filling → Processing → Storage
(< 0.5mm) *(185°F)* *(212°F; 45 min)*

Frozen

Mangoes → Cutting Mill → Pulper → Blanching → Cooled → Filling →
(0.033 in.) *(194–199°F; 1 min)* *(90–100°F)*
Freeze
(–11°F)

Mango Juice

water
Mango puree → Mix → Heat Exchanger → Filling → Processing →
(12 –15 Brix; 0.4–0.5% TA) *(185°F)* *(401 × 411)* *(212°F); 20 min)*

Cooling → Packed → Storage

Mango Concentrate

Mango puree → Pasteurize → Cool → Centrifuge → Pulp
(167°F; 1 min) *(500 rpm)* Serum → Evaporated
Freeze Concentrate
(45 Brix)

Mango Slices/Scoops (canned)

Mango → Slice/scoop → Fill → Exhaust → Process → Seal → Store

Aseptic Packed Mango Beverage

Mango puree → Mix → Aseptic Filling → Seal → Cool
(110°C;45–60 s) *(104°F)*

Mango Nectar

Mango Puree → Filtration → Processing → Packing
(vacuum, flash, aseptic)

Mango Leather

Mango Puree → Finisher/Sieve → Spread → Dry
(30–50 mesh) (50–80°C; 2–20 h/layer)

Mango Powder

Mix → Drum-Dry → Flakes → Mill → Powder

Mango puree → Mix → Heat → Homogenize → Atomizer → Spray-Dry →
Cool → Pack *(2800 rpm)* *(2800 rpm)* *(329°F)*
(40°F, 15 min)

Figure 36.7 *Processing of selected mango products. Adapted from Woodroof 1975; Nagy and others 1993; Nakasone and Paull 1998; Narain and others 1998; Taylor 2001.*

TABLE 36.7 Commercially Processed Mango Products.

Product	Characteristics	Packaging	Shelf-Life	Applications
Slices[b]	Peeled; sectioned; ready-to-eat	Resealable 24 oz glass mason jar; 64 oz. glass	Refrigeration	Snacks; dessert
IQF dices[c]	3/8 in.; 5/8 in.; $\frac{3}{4}$ in.; 1 in.	33 lb carton	Storage below 0°F	Fruit preparations; bakery products; frozen desserts
Aseptic puree[c]	Brix: 14–19°	55 gal drums; 6 gallon carton	Shelf-stable	Fruit preparations; beverages; dairy products; bakery products; frozen desserts
Aseptic puree[c] (organic)	Brix: 14–16°	55 gal drums; 6 gallon carton	Shelf-stable	
Frozen Puree[c]	Brix: 15–17°	430 lb drum	Storage below 0°F	
Aseptic concentrate[c]	Brix: 28°min	55 gal drums; 6 gallon carton	Shelf-stable	Beverages; dairy products
Frozen concentrate[c]	Brix: 28°min	463 lb drum	Storage below 0°F	
Clarified concentrate[c]	Brix: 70°	55 gal drums	Storage below 0°F	
Flakes[a]	Moisture $\leq$3.0%	22.1 lb bag-in-box; 24.5 lb per box; polyethylene bag;	18 months (70°F)	Dry fruit preparations; substitute for fresh mango puree (6 : 1 reconstitution); beverages; bakery products; frozen desserts, and soft food diets
Powder[a]	Moisture $\leq$3.0%	39.6 lb bag-in-box 41.8 lb polyethylene bag	18 months (70°F)	Dry fruit preparations; beverages; bakery products; substitute for mango puree(6 : 1 reconstitution); fruit preparations; dairy products; soft food diets;frozen desserts
Infused dried diced[d]	Moisture 12% $\pm$ 3%; Oil <1%	25 lbs 10 lbs	Store in a cool(preferably 40–50°F), dry location. Shelf-life : 1 year	Cereals; trail mixes; bakery products; snacks
Essence[c]		40 lb pail		Applications in water-based system to impart mango flavor

Sources: [a]Trobana® Products, Confoco, http://www.confoco.com/confoco/ingles/mango.htm; [b]Del Monte http://www.delmonte.com/Products/FruitItem.asp?id=46; [c]iTi Tropicals, http://www.ititropicals.com/ProductList.pdf; [d]GracelandFruit, http://www.gracelandfruit.com/dried_fruit.php

information of commercial mango puree. Frozen mango puree is usually vacuumed to remove air (Wu and others 2005). Mango pulp in trays has been frozen using air-blast freezers or plate freezers (−34.9°F to −40°F) and stored at −0.4°F to −4.0°F prior to consumption or export. Prior to a quick freezing process, mangoes are subjected to a "steaming" tunnel, cooled, peeled and passed through a combined pulper/destoner unit. The resulting puree is homogenized, sterilized, placed in an aseptic buffer tank, cold sterilized, and filled into bags-in-drums (Pruthi 1999).

36.3.5.2 Mango Juice Concentrate. Mango concentrates are utilized in the preparation of juice and energy drinks (Fig. 36.7; Table 36.7). Enzymatic liquefaction has been employed to improve the quality of higher °Brix concentrates (Wu and others 2005).

36.3.5.3 Mango Juice. Figure 36.7 outlines the processing steps for mango juice production. Studies have been conducted showing the use of enzymes to facilitate juice extraction, control cloud stabilization and viscosity, and increase the free flow and yield extraction of juice (Occeña-Po 2006).

Mango juice can also be aseptically packaged in a bag-in-box and stored at ambient temperature, although storing at low temperature preserves it better (Wu and others 2005).

Mango juice can be made into mango nectar or squash, and processed by any of the following methods:

1. Spin-cooker;
2. Flash pasteurization; or
3. Pasteurization and aseptic packing in plastic-lined cartons (Wu and others 1993, 2005). When a preservative (350 ppm SO_2 or 0.1% sodium benzoate) is utilized, the product is referred to as mango squash.

36.3.5.4 Mango Slices and/or Scoops. Figure 36.7 summarizes the process for canning mango slices/scoops. Mango slices can also be packed in polythene or pliofilm bags and stored at −0.4°F for 12–18 months. Calcium pretreatment (2%) helps keep the texture firm. The advantages of quick freezing of mango slices by immersion in liquid nitrogen are reduced weight loss and lower loss in textural properties (Pruthi 1999).

36.3.5.5 Mango Leather and Mango Bar. Mango leather is a promising source of vitamin A (provitamin A content of 600–650 retinol equivalents). The industrial preparation of mango leather is summarized in Figure 36.7. Highly acceptable mango bars have been prepared using 25°Brix pulp with 0.5% sodium alginate and drying at 140°F (Singh and others 2004). The drying rate for mango leather was reported to have been lowered by the addition of soy protein concentrate (Gujral and Khanna 2002).

Sulfiting is a common practice in developing countries to retain the yellow-orange color of dehydrated mango products, but has been under critical consideration with respect to allergen labeling of foods. The process for preparing infused-dried mango fruit (Fig. 36.7) retains the natural color without the use of sulfites (Sinha 1988).

36.3.5.6 Other Mango Products. Figure 36.7 outlines the manufacture of other mango products. Table 36.7 summarizes the characteristics and applications of commercial mango products.

36.4 PINEAPPLE

36.4.1 Introduction

36.4.1.1 Historical Brief. It is believed that the pineapple originated in southern Brazil and Paraguay, and was spread by the Indians to other parts of South and Central America. The pineapple was brought to Europe by Columbus in 1493, and distributed to the Pacific Islands, India, Philippines, and Africa by the Spanish and Portuguese explorers.

Hawaii used to produce most of the world's pineapple with the establishment of the first commercial plantation on Oahu in 1885. However, since 1960, Hawaii's pineapple industry has been on the decline. Pineapple was first canned in Malaysia and canned fruits were exported from Singapore around 1900 (Rieger 2005).

36.4.1.2 Classification. The pineapple fruit is a multiple fruit covered with a waxy, leathery rind made up of hexagonal "eyes" arranged spirally, denoting the position of individual flowers. The predominant cultivar is *Smooth Cayenne*, chosen for worldwide dissemination for its desirable characteristics. Table 36.8 summarizes the characteristics of pineapple cultivars used for canning. The Del Monte Gold® Extra Sweet, reportedly the world's sweetest pineapple, claims to be twice as sweet as the traditional variety, and contains up to four times the vitamin C.

36.4.1.3 Cultivation. Pineapples can take up to 18 months to grow. They are grown in climates with high rainfall and grow best in well-drained sandy loams with pH 4.5–6.5, and temperatures of 75–90°F.

36.4.2 Harvest, Postharvest, and Storage

Pineapple is categorized as a nonclimacteric fruit, not capable of continuing its ripening once removed from the plant. The most common method of determining maturity of the pineapple fruit is by the change in color from green to yellow, the flat eyes, and the large, well-formed crown. However, a minimum Brix of 12 measured by a refractometer can signal harvest maturity. Pineapples for canning are allowed to reach a more advanced stage (about $\frac{1}{2}$ to $\frac{3}{4}$ yellow) prior to harvest. An application of ethephon during ripening of the pineapples forces synchronization of maturity and eliminates multiple pickings.

Fruits for export are almost completely green when harvested for trans-oceanic shipment, but pineapples that are too ripe and yellow are hand-harvested for the fresh market. In big plantations, mechanical harvesting is utilized, with two conveyors, one on top of the other, breaking off the pineapples and carrying the fruits to the lower conveyor where they are de-crowned (Fig. 36.8). Fresh pineapples are washed and waxed prior to packing in boxes.

Pineapples can be stored for up to 4 weeks at temperatures $\geq$45°F. The recommended storage for pineapple is 45–54°F, and 90–95% relative humidity. (Paull and Chen 2003).

TABLE 36.8 Commercial Pineapple Varieties.

Group	Varieties	Country	Flesh Color	Sugar (Brix)	Acid (%TA)	Descriptor
Cayenne	Smooth Cayenne, Hilo, Kew, Champaka, Sarawak	Thailand, Philippines, Indonesia, Australia	Pale yellow	12–16	0.5–0.9	Most important group; more than 70% of pineapple grown in the world; ave. wt. 2.5 kg; for fresh fruit consumption and canning
Queen	Moris, Mauritius, MacGregor, Ripley Queen, Alexandra	India, South Africa, Australia, South East Asia	Deep yellow	14–18		Ave. wt. 0.8–1.5 kg; fresh fruit
Spanish	Singapore Spanish, Ruby, Red Spanish, Masmerah, Gandul, Hybrid 36, Selangor Green, Nangka, Betik	Malaysia	Deep golden yellow	10–12	0.3–0.6	Ave. wt. 1–2 kg; poorer cannery recovery; fibrous flesh
Pernambuco and Mordilona	Perolera	Brazil, Ecuador, Peru, Colombia	White	Mild flavor	Low acid	Less economic importance, restricted to South America; Perolera has high vitamin C content and is used in hybridization programs for improving Smooth Cayenne cultivars

Source: Shukor and others (1998).

36.4.3 Supply and Demand

36.4.3.1 Supply. Table 36.9 summarizes the average world production of pineapple over the last five years (2001–2005). World production in 2005 was 15,886,647 metric tons over 857,771 hectares. The top 10 leading producers contributed about 75% of world production. Pineapple production and marketing in the Philippines (and Costa Rica, Honduras, and Ecuador) is conducted exclusively by multinational corporations,

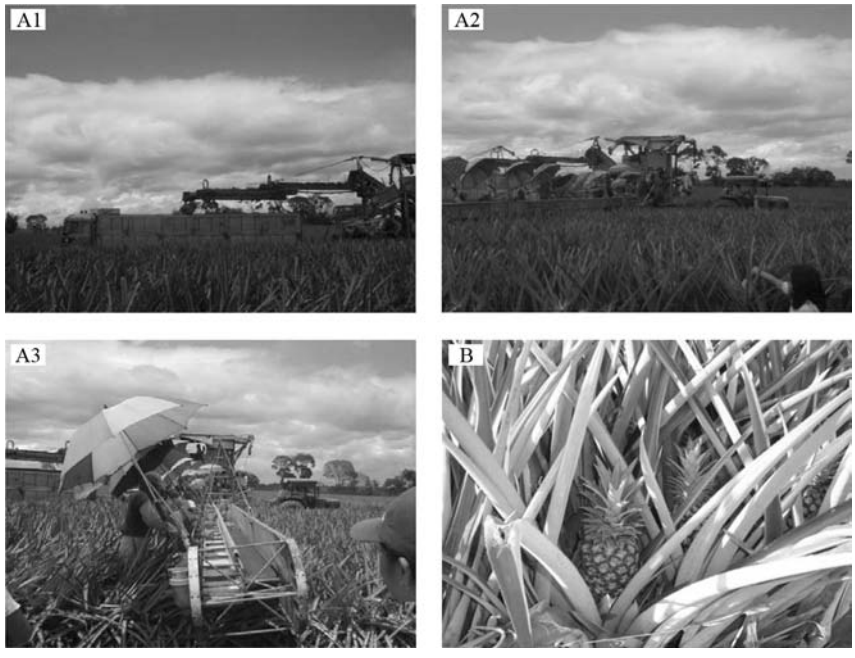


Figure 36.8 *(A1–3) Plantation harvesting, sorting, and decrowning, (B) Pineapple fruit plant.*

TABLE 36.9 The Ten Leading Countries and World Aggregate in Pineapple Production (2001–2005).

	Production (Mt)		Area Harvested (Ha)	
Country	Average (2001–2005)	2005	Average (2001–2005)	2005
1. Thailand	1,952,709	2,050,000	86,209	90,000
2. Philippines	1,702,863	1,800,000	46,785	49,000
3. China	1,326,900	1,460,000	68,564	75,400
4. Brazil	1,431,468	1,418,420	57,896	53,116
5. India	1,262,000	1,300,000	86,000	90,000
6. Nigeria	887,400	889,000	115,800	116,000
7. Costa Rica	898,570	725,224	15,956	17,400
8. Mexico	689,695	720,900	16,624	17,906
9. Indonesia	622,126	673,065	67,400	80,000
10. Kenya	566,242	600,000	13,212	13,500
World	15,442,696	15,886,647	827,983	857,771

Source: FAO (2006).

on large plantation systems with mechanized operations. In contrast, pineapple production in Thailand is through thousands of small farms.

The top three suppliers of fresh and frozen pineapple to the United States are Costa Rica, Honduras, and Ecuador. Although the Philippines is the largest supplier of processed canned pineapple products and juice, it has exhibited a slight decline in shipments in recent years.

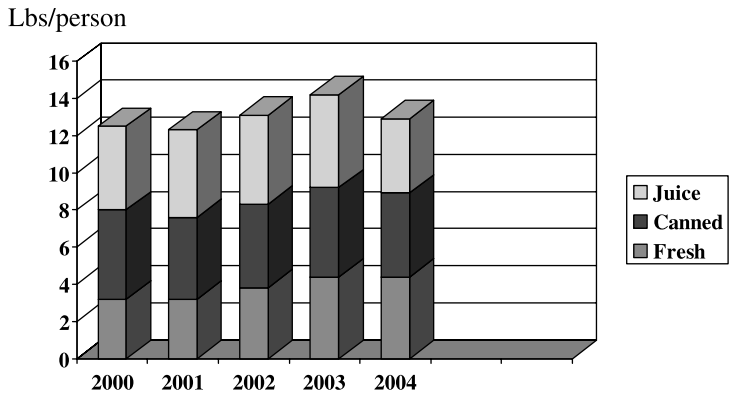


Figure 36.9 *U.S. per capita consumption of pineapple 2002–2004.*

36.4.3.2 Demand. Figure 36.9 summarizes U.S. per capita consumption of fresh and processed pineapple products for the period 2002–2004. Like other tropical fruits, total pineapple consumption in the United States declined (4.33 lb per person) slightly in 2005.

36.4.4 Physico-Chemical, Nutritional, and Phytochemical Quality

36.4.4.1 Physico-Chemical Quality. Ripe mature pineapple flesh contains about 85% moisture, its pH, % citric acid, and Brix are, 3.4, 0.70, and 14.0, respectively. It contains about 7% sucrose and 3% each of glucose and fructose. Citric acid is the predominant organic acid followed by malic, oxalic, and phosphoric acid.

36.4.4.2 Flavor Components in Pineapple. The characteristic pineapple flavor is due to two thioesters, methyl 3-(methylthio) propanoate and ethyl 3-(methylthio) propanoate (Brat and others 2004). In addition to these compounds, Elss and others (2005) also reported methyl 2-methylbutanoate, methyl butanoate, methyl hexanoate, ethyl hexanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone(mesifurane) and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) as major constituents (Table 36.11).

36.4.4.3 Nutritional Composition. Table 36.3 shows the nutritional composition of fresh pineapple and processed pineapple products. Pineapple is a low-calorie fruit. It contains little sodium and fat. It is a good source of potassium and vitamin C. It also contains vitamin A.

36.4.4.4 Phenolic Content and Antioxidant Compounds. Phenolics in pineapple have been identified as *p*-coumaric acid and ferulic acid (33–73 and 20–76 μg/g fresh weight, respectively) (Dull 1971). Wu and others (2004) reported total phenolics and total antioxidant capacity of 1.74 mg GAE/g and 7.93 μmol TE/g, respectively, in pineapple (Table 36.3). Huang and others (2004) reported total phenolic content in pineapple core to be 74.42 ± 3.31 μg of gallic acid equivalents/g fresh weight, and antioxidant activity (73.05 ± 3.95 μmol of ascorbic acid/g fresh weight). The alkaloid 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-*â*-carboline, acting as antioxidant, was reported by Herraiz and Galisteo (2003) in pineapple (0.62 μg/g ± 1.02) and its juice (1.69 μg/g ± 1.40).

TABLE 36.10 Nutritional Value of Pineapple and Pineapple Products.

Nutrients/100 g	Raw[a] Pineapple, (All Varieties)	Pineapple Juice[b], Canned with Ascorbic Acid	Pineapple Canned Juice Pack[c], Solids and Liquids	Pineapple Frozen, Chunks[d], Sweetened	Pineapple Juice, Frozen Concentrate[e], Unsweetened, Undiluted	Infused-Dried[f] Pineapple
Calories (kcal)	48.0	56.0	60.0	86	179	341
Total fat (g)	0.12	0.08	0.08	0.10	0.10	1.31
Saturated fat (g)	0.009	0.005	0.006	0.007	0.007	0.2
Polyunsaturated fat (g)	0.042	0.028	0.029	0.035	0.035	0.8
Monounsaturated fat (g)	0.014	0.009	0.010	0.012	0.012	0.3
Cholesterol (mg)	0.0	0.0	0.0	0.0	0.0	<0.1
Sodium (mg)	1.0	1.0	1.0	2	3	32.0
Potassium (mg)	115.0	134.0	122.0	100	472	228.0
Total carbohydrate (g)	12.63	13.78	15.70	22.20	44.30	85.4
Total fiber (g)	1.4	0.20	0.80	1.1	0.7	5.1
Total sugar (g)	9.26	13.58	14.45	21.10	43.60	66.1
Protein (g)	0.54	0.32	0.42	0.40	1.30	0.39
Calcium (mg)	13.0	17.0	14.0	9	39	16.0
Iron (mg)	0.28	0.26	0.28	0.40	0.90	0.90
Vitamin C (mg)	36.2	24.0	9.5	8.0	42.0	350.0
Vitamin A (IU)	56.0	5.0	38.0	30	50	70.0

Sources of data for: [a,b,c,d,e]: http://www.ars.usda.gov/nutrientdata

[f]Data of commercial products. Infused with sugar prior to drying, contains added ascorbic acid and high oleic sunflower oil. Courtesy of Graceland Fruit Inc, Frankfort, MI, USA.

36.4.5 Pineapple Processing

Pineapples harvested from the field are mechanically graded for size, automatically peeled, cored, and trimmed using a machine referred to as *Ginaca*. The resulting pineapple cylinder is further divided into sections including slices, chunks, and tidbits, and canned. Figure 36.10 represents the primary pineapple products and byproducts. For every 100 lb of pineapple fruit, only about 27% goes into the processing of primary pineapple products, of which 59% are processed as pineapple chunks, slices, and tidbits; 41% goes into the processing of crushed pineapple and/or utilized as juice material. About 35% of the pineapple fruits are utilized as juice material, 91% of which is made into pineapple juice, and 9% is the remaining pomace after extraction. About 38% of the pineapple fruits become byproducts, including pomace (32%) and mill juice (68%). Figure 36.11 shows photographs of various commercial processed pineapple products derived from fresh pineapple. Figure 36.12 provides flow diagrams for the processing of frozen and dehydrated pineapple products. Table 36.12 summarizes high-sugar pineapple products including jams, marmalades, and conserves, which are increasingly popular food toppings.

36.4.5.1 Selected Pineapple Products

Pineapple Slices. The USDA/FDA Standards for grades and identity, quality, and container fill weight provide guidelines for canned pineapple products. Pineapple fruits

TABLE 36.11 Aroma Profile of Pineapple Fruit and Pineapple Products.

Aroma Compound	Juice from Fresh-Cut Pineapple (μg/L)	Single-Strength Juices (μg/L)	Concentrates (μg/kg)	Juice from Concentrates (μg/L)	Water Phases/ Recovery (mg/L)
Methyl 2-methylbutanoate	1500	180		2	85.0
Ethyl 2-methylbutanoate	190	25		10	45.0
Methyl hexanoate	1300	200		3	220.0
Ethyl hexanoate	500	25		2	145.0
Methyl 3-(methylthio)-propanoate	1500	550	40	12	112.0
Ethyl 3-(methylthio)-propanoate	470	130		12	109.0
2,5-Dimethyl-4-methoxy-3(2H)furanone	1500	110		1	10.0
γ-Butyrolactone	100	75	60	33	
Methyl 3-hydroxyhexanoate	205	44		2	5.1
Ethyl 3-hydroxyhexanoate	75	27		1	2.1
γ-Hexalactone	715	230	40	22	9.9
Methyl 3-acetoxyhexanoate	460	50		6	7.8
Ethyl 3-acetoxyhexanoate	60	13		1	2.4
δ-Hexalactone	250	80	40	15	0.6
γ-Octalactone	90	70		2	6.4
δ-Octalactone	100	31		4	0.0
2,5-Dimethyl-4-hydroxy-3(2H)furanone	960	1000	1600	305	1.1

Source: Elss and others (2005).

are sized and trimmed mechanically to fit corresponding can sizes. Slicers equipped with single or multiple blades cut slice packs out of trimmed pineapple cylinders. Slices or rings of $\frac{1}{2}$ in. are designated for No. $2\frac{1}{2}$ cans, and 25/64 in. slices for No. 2 cans. Pineapple slices are visually graded to meet specifications, and manually or automatically packed in cans. Slices are graded as *fancy* (geometrically perfect, excellent, uniform color, whole), *choice cut* (not as perfect with less color), or *standard*. The cans go to an exhaust or vacuuming chamber, or directly to the syruping step. The syrup concentration depends on the quality

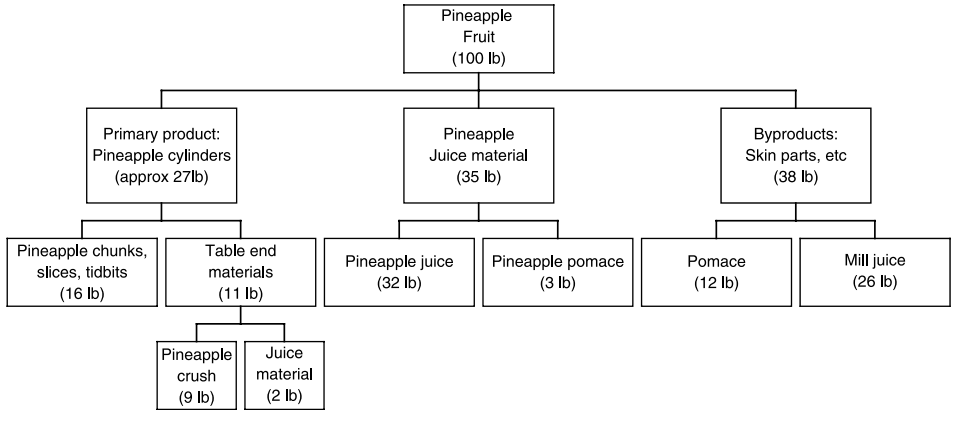


Figure 36.10 *Primary pineapple products and by products. (Adapted from Mumaw 1996).*

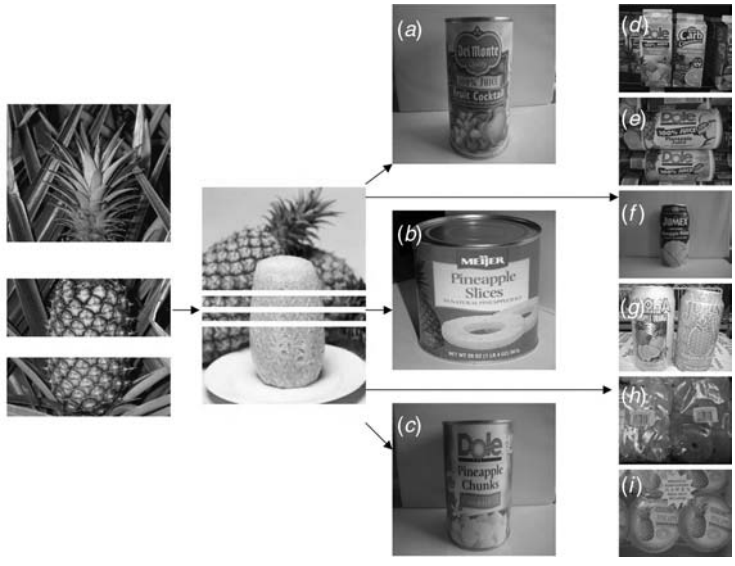


Figure 36.11 *Pineapple products processed from fresh pineapple (a)-fruit cocktail; (b)-slices; (c)-chunks; (d)-juice blends; (e)-concentrate; (f)-Nectar; (g)-Juice Drinks; (h)-Dehydrated Slices; (i)-Custard.*

of the pineapple product: the Hawaiian No. 1 grade is packed with 24°Brix cut-out syrup, and No. 2 and 3 grades with 20°Brix syrup. Atmospheric closure is used for hot filling (190.4°F). With a prevacuuming syruper, either a mechanical vacuum or steam-flow closure is utilized. Processing is carried out by either a continuous rotary pressure sterilizer (215.6–219.2°F) or by an ordinary retort operating at atmospheric pressure (212°F for 12–18 min for No. $2\frac{1}{2}$ cans). Cans from a rotating cooker go to a rotating cooler (Pruthie 1999; Hepton and Hodgson 2003).

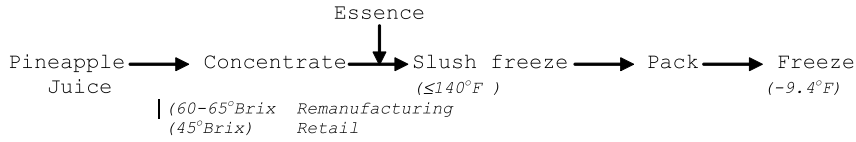
Pineapple Chunks and Tidbits. Pineapple slices may be further cut into tidbits, cubes, and chips, and thicker slices are cut into chunks or pieces. For pineapple tidbits, fresh pineapple is cut into segments (1.27 cm × 1.37 cm), in symmetrical segments or cubes (Woodroof and Luh 1975).

Pineapple Juice. The extraction of juice is often combined with the production of canned fruit packs to utilize any pineapple pieces discarded from other product lines, including slices that have been cut too thick or too thin, and broken pieces. These are all crushed to extract juice. Other solid components utilized for juice consist of

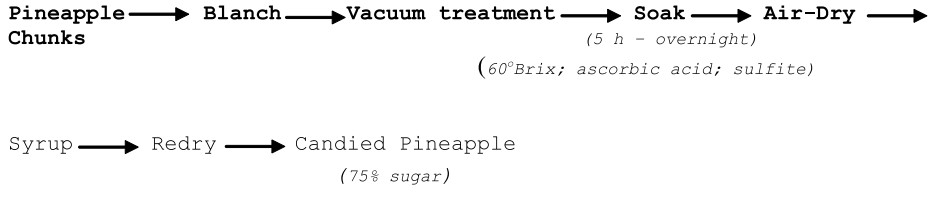
1. Pineapple cores;
2. *Eradicator meat* (thin layer of flesh between the shell and fruit cylinder removed using *Ginaca*);
3. Trimmings.

Whole fruits not required for solid packs, or fruits too small for canning are peeled and pressed for juice, or simply crushed and pressed. The juice is homogenized to stabilize

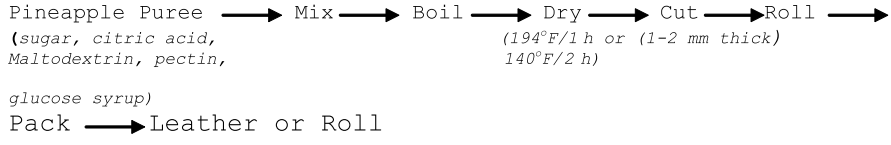
Frozen Pineapple Concentrate



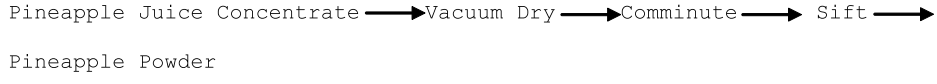
Candied Pineapple



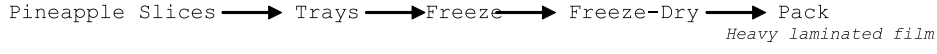
Pineapple Leather and Roll



Pineapple Powder



Freeze-Dried Pineapple



Intermediate-Moisture (IMF) Pineapple

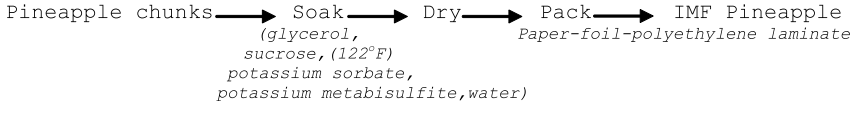


Figure 36.12 *Processing of selected pineapple products.*

the slightly cloudy appearance, and heated to coagulate solids. The thin slurry passes through a continuous centrifuge, removing suspended solids, including fibers and coarse pieces (Woodroof and Luh 1975; Hepton and Hodgson 2003). "Processing beverage juice" (juice from fruit, cores and skin waste) is distinguished from "skin juice", utilized

TABLE 36.12 High-Sugar Pineapple Products.

Products	Ingredients	Procedure	Comments
Pineapple jam	Pineapple puree or pulp; sugar	1. Boil the pineapple pulp or puree with sufficient quantity of sugar until it reaches a thick consistency. 2. Pack in jars. 3. Process at 100°C for 15 min. 4. Cool immediately.	It can be prepared from a single fruit or using a combination of different fruits, e.g., pineapple–papaya (30 : 70); mango–pineapple.
Pineapple conserve[a]	Crushed pineapple; orange or lemon juice; sugar; raisins; nuts	1. Mix crushed or chopped pineapple with orange or lemon juice. 2. Heat until the mixture becomes thick. 3. Pack in jars. 4. Process at 100°C for 15 min. 5. Cool immediately.	Nuts are optional; can mix with other fruits like mango.
Pineapple marmalade	Crushed pineapple; lemon zest; sugar	1. Mix crushed or chopped pineapple with sugar. Add the lemon zest. 2. Heat until the mixture becomes thick. 3. Pack in jars. 4. Process at 100°C for 15 min. 5. Cool immediately.	In lieu of lemon zest, finely chopped orange rind.
Pineapple confectionery jelly[b]	Pineapple juice; granulated sugar; glucose syrup; citric acid	1. Mix pineapple juice and pulp with granulated sugar and glucose syrup (75%). 2. Citric acid is added to reduce the pH of the jellies to avoid pectin degradation, premature setting, and to reduce the rate of sucrose inversion. 3. Maintain temperature at not less than 90°C immediately after adding acid. 4. The product is packed in laminated oriented polypropylene/polyethylene package.	

Sources: [a]Woodroof and Luh (1975); [b]Shukor and others (1998).

as a base for sweeteners. However, the use of other solid pineapple materials for juice production is not allowed in the United States (Shukor and others 1998; Hepton and Hodgson 2003).

Crushed Pineapple. Crushed pineapple is obtained from finely cut or shredded pieces of pineapple from other processing lines, from small, irregularly-shaped slices unsuited for regular canned pineapples, and eradicator meat. The crushed pineapple is pumped into steam-jacketed kettles and heated to 170.6–195.8°F, automatically packed into cans, sealed, and heat processed for 10 min. An alternative processing is to cold-fill, exhaust to 179.6°F, seal and process in boiling water for 30–40 min (Woodroof and Luh 1975; Pruthie 1999).

Minimally Processed Pineapple

"FRESH CUT" PINEAPPLES. The market for minimally processed pineapple has increased. "Fresh cut" pineapples are packaged as whole, slices, or chunks in sealed plastic tubs or cups, and chilled to optimum temperature levels until purchased by the consumer. The balance between sweetness and acidity is relevant in selecting the pineapple cultivar to use. Fresh-cut processing causes wounding, increases metabolic activities, and decompartmentalizes enzymes and substrates, causing possible browning and decay of the fresh fruit. Gonzalez-Aguilar and others (2004) reported that treatment of pineapple slices with anti-browning agents isoascorbic acid (IAA; 0.1 mol/L), ascorbic acid (AA; 0.05 mol/L), or acetyl cysteine (AC; 0.05 mol/L), prolonged the shelf-life of fresh-cut pineapples for up to 14 days at 50°F, with no observed off-flavors. Martinez-Ferrer and Harper (2005) reported the use of methyl jasmonate (MJ) emulsion (10^{-4} M) decreased microbial growth in diced pineapple by 3 logs after 12 days storage at 44.6°F, without affecting firmness or color.

HIGH PRESSURE PROCESSING (HPP). High hydrostatic pressure is a novel technology for minimal processing of pineapple products. Bacterial survival and total yeast and fungi counts decrease with increase in processing pressure in fresh-cut pineapple chunks packed in heat-sealed polyethylene pouches and treated under various ultra-high pressure, temperature, and time combinations (Hepton and Hodgson 2003). Water and solute of pressure-pretreated pineapple have been reported to demonstrate a significantly higher diffusion rate during osmotic dehydration (Ramaswamy and others 2005).

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37

Fruit Juices

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37.1 Introduction 847
37.2 Production of Flavor Compounds during Fruit Maturation 851
37.2.1 Citrus Fruit 852
37.2.2 Apples and Pears 853
37.2.3 Grapes 855
37.3 Processing of Fruit Juice 855
37.3.1 Juice Extraction 856
37.3.2 Post-Extraction Juice Treatments 856
37.3.3 Thermal and Non-Thermal Processing Treatments 856
37.3.3.1 Pasteurization 857
37.3.3.2 Canning 857
37.3.3.3 Irradiation 857
37.3.4 Juice Concentration 858
37.4 Packaging of Fruit Juices 859
37.4.1 Characteristics of Plastic Packaging Materials 859
37.4.2 Aseptic Packaging 860
37.5 Storage of Fruit Juices 861
37.6 Challenges for the Fruit Juice Industry 862
References 862

37.1 INTRODUCTION

Fruit juices represent a growing category in the United States beverage market. In 2000, juice consumption accounted for 44% of total fruit consumption, and this percentage is expected to increase in the future. Orange juice is the most popular of the juice products,

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accounting for 60% of all juice consumed. Although orange, grapefruit, apple, and grape juices are considered to be the more traditional fruit juices, fruit juice blends of traditional and nontraditional fruits and fruit drinks contribute significantly to the fruit juice market and have received wide consumer acceptance (Thor and Savitry 2004).

The flavor of fruit juices is developed through enzymatic reactions during the maturation of the fruit and influenced by the processing the juice undergoes following extraction. The volatile compounds responsible for the characteristic flavor of fruits are dependent not only on the type of fruit, but also the cultivar. The flavor descriptions, based on gas chromatography-olfactometry, of volatile compounds identified in fruit juices are compiled in Table 37.1. The data presented in this table reflect the significant amount of research conducted on the flavor characteristics of citrus juices. However, it is also interesting to note that many volatile flavor compounds are present in several different fruit juices. Esters are major contributors to the floral, fruity aromas of many juices.

TABLE 37.1 Flavor Characteristics of Volatile Flavor Compounds in Fruit Juices.

Volatile Compound	Flavor Characteristic	Source[a]
	Esters	
Ethyl acetate	Fruity, orange	Orange juice (G, I), grapefruit juice (D), muscadine grape juice (A)
Isobutyl acetate	Solvent-like	Apple juice (B)
Octyl acetate	Unpleasant, dusty, green	Orange juice (I)
p-menth-1-en-9-yl acetate	Citrusy, fruity, floral	Orange juice (I)
Ethyl propanoate	Fruity	Orange juice (G), grapefruit juice (D)
Methyl butanoate	Fruity	Orange juice (I)
Ethyl butanoate	Fruity, green, floral, orange	Orange juice, (C, G, I), grapefruit juice (D, F), apple juice (B), muscadine grape juice (A)
Propyl butanoate	Cooked apple-like, fruity, musty	Orange juice (C), apple juice (B)
Ethyl 2-methylpropanoate	Fruity, sweet	Orange juice (G), grapefruit juice (D, F, H)
Ethyl 2-methylbutanoate	Cooked apple-like, green apple, fruity	Orange juice (G), grapefruit juice (D, H), apple juice (B), muscadine grape juice (A)
Ethyl hexanoate	Fruity, green apple, orange	Orange juice (G, I), grapefruit juice (D, F), muscadine grape juice (A)
Methyl octanoate	Mint/citrus/moldy/musty/oil	Orange juice (C)
Ethyl decanoate	Roasted meat/cooked apple sauce	Orange juice (C)
Ethyl 3-hydroxybutanoate	Hay-like, burnt marshmallow, muscadine	Muscadine grape juice (A), grape musts (E)
Ethyl 3-hydroxyhexanoate	Sweet, fruity	Orange juice (G), grapefruit juice (D, F)
	Terpenoid Compounds	
α-Pinene	Pine-tree, citrus, spicy, woody	Orange juice (G, I), grapefruit juice (D, F)
γ-Carene	Floral	Orange juice (I)
Carvone	Musty, floral, minty	Orange juice (C)
β-Cubebene	Floral, terpene-like, lemon	Orange juice (I)
β-Damascenone	Sweet, honey	Grapefruit juice (H)

(*Continued*)

TABLE 37.1 *Continued.*

Volatile Compound	Flavor Characteristic	Source[a]
Geraniol	Spicy, rosy, floral	Orange juice (I), muscadine grape juice (A)
β-Ionone	Violet-like	Orange juice (G, I)
Limonene	Citrus, minty, earthy, fruity, lemon, anise	Orange juice (C, G, I), grapefruit juice (D, F, H)
Linalool	Flowery, musty, incense, fruity, lemon	Orange juice (C, G, I), grapefruit juice (D, F, H)
β-Myrcene	Mossy, piney, earthy, citrus, geranium	Orange juice (C, G, I), grapefruit juice (D, F)
Nootkatone	Grapefruit	Grapefruit juice (D, H)
1,10-Dihydo-nootkatone	Grapefruit	Grapefruit juice (H)
β-Pinene	Citrus, terpene-like	Orange juice (I)
Terpinen-4-ol	Apple	Grapefruit juice (H)
	Acids	
Acetic acid	Sour, pungent, vinegar	Orange juice (G), grapefruit juice (D), muscadine grape juice (A),
2-Methyl propanoic acid	Buttery/cooked rice	Orange juice (C)
Butanoic acid	Sweaty, rancid	Orange juice (G), grapefruit juice (D)
2- and 3-methylbutanoic acid	Sweaty, dried fruit	Grapefruit juice (D), muscadine grape juice (A)
Hexanoic acid	Fruity, fatty, sweet	Orange juice (I)
Octanoic acid	Moldy, unpleasant	Orange juice (I)
	Alcohols	
3-Ethoxy-1-propanol	Fruity/floral	Orange juice (C)
2- and 3-methylbutanol	Malty, bitter chocolate	Orange juice (G), grapefruit juice (D), muscadine grape juice (A)
1-Hexanol	Floral	Orange juice (I)
trans-3-hexenol	Green, woody, benzene-like	Orange juice (I), grape musts (E)
1-Octanol	Grapefruit, herbal	Orange juice (I)
1-Nonanol	Fruity, floral	Orange juice (I)
trans, cis-2,6-nonadienol	Cucumber, melon	Muscadine grape juice (A), grape musts (E)
2-Phenylethanol	Rosy, plastic, styrene	Muscadine grape juice (A)
	Aldehydes	
Acetaldehyde	Fruity, pungent	Orange juice (I), grapefruit juice (F)
2- and 3-methylbutanal	Malty	Orange juice (G)
Hexanal	Grassy, green, fruity, orange floral	Orange juice (G, I), grapefruit juice (D, F), apple juice (B), grape musts (E), muscadine grape juice (A)
cis-3-hexenal	Green, grassy, medicine, musty, fruity	Orange juice (C, G), grapefruit juice (D, F, H)
Octanal	Green, citrus-like, fresh, minty	Orange juice (G), grapefruit juice (D, F, H)
trans-2-hexenal	Green, apple-like	Grapefruit juice (H), apple juice (B)
trans-3-hexenal	Grass, grape	Grape musts (E)
Nonanal	Citrus-like, soapy, musty, floral	Orange juice (C, G, I), grapefruit juice (D, F, H)

(*Continued*)

TABLE 37.1 *Continued.*

Volatile Compound	Flavor Characteristic	Source[a]
cis-2-nonenal	Fatty, green, geranium	Orange juice (G), grapefruit juice (D, H)
trans-2-nonenal	Fatty, tallowy, soapy, musty, floral	Orange juice (C, G), grapefruit juice (D, F, H)
trans, cis-2,4-nonadienal	Geranium	Grapefruit juice (H)
trans, trans-2,4-nonadienal	Fatty, fried	Grapefruit juice (H)
trans, cis-2,6-nonadienal	Cucumber	Orange juice (G), grapefruit juice (H), muscadine grape juice (A)
Decanal	Green, soapy, musty, fruity, lemon	Orange juice (C, G, I), grapefruit juice (D, F)
cis-2-decenal	Soapy	Grapefruit juice (H)
trans-2-decenal	Geranium	Grapefruit juice (H)
trans, cis-2,4-decadienal	Geranium	Grapefruit juice (H)
trans, trans-2,4-decadienal	Fatty, waxy, fried	Orange juice (G), grapefruit juice (F, H)
4,5-epoxy-*trans*-2-decenal	Metallic	Grapefruit juice (D, H)
trans-2-undecenal	Geranium	Grapefruit juice (H)
	Ketones	
2-Propanone	Fruity	Orange juice (I)
2-Methyl-3-buten-2-one	Buttery, cannel	Grape musts (E)
2,3-Butanedione	Caramel, buttery, cream cheese	Orange juice (G), grapefruit juice (D), muscadine grape juice (A), grape musts (E)
1-Penten-3-one	Etheral, pungent	Orange juice (G), grapefruit juice (D)
1-Hepten-3-one	Geranium-like	Grapefruit juice (D, F)
6-Methyl-1-heptenone	Grass, green	Grape musts (E)
1-Octen-3-one	Mushroom, woody, earthy	Orange juice (G), grapefruit juice (D, F, H), grape musts (E), muscadine grape juice (A)
cis-1,5-octadien-3-one	Geranium	Orange juice (G), grapefruit juice (D, H)
γ-Decalactone	Coconut	Grapefruit juice (H)
Winelactone	Sweet, spicy	Grapefruit juice (F)
	Furans	
2-Methyl-3-furanthiol	Meaty	Grapefruit juice (H)
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Caramel-like	Orange juice (G), grapefruit juice (D, H), muscadine grape juice (A)
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3H)-benzofuranone	Sweet, spicy	Grapefruit juice (D), orange juice (G)
	Pyrazines	
2-Isopropyl-3-methoxypyrazine	Earthy, beany	Orange juice (G)
3-Isopropyl-2-methoxypyrazine	Earthy, beany	Grapefruit juice (D)
	Phenolic Compounds	
o-Aminoacetophenone	Foxy, cat urine	Muscadine grape juice (A)
Phenethyl acetate	Sweet, pipe tobacco	Muscadine grape juice (A)

(*Continued*)

TABLE 37.1 *Continued.*

Volatile Compound	Flavor Characteristic	Source[a]
Phenylacetaldehyde	Caramel, syrup, honey-like, floral	Grapefruit juice (D), muscadine grape juice (A), grape musts (E)
p-Vinylguaiacol	Curry powder, musty	Orange juice (C), muscadine grape juice (A)
Vanillin	Vanilla	Orange juice (G), grapefruit juice (D, F, H)
	Sulfur-Containing Compounds	
p-1-Menthene-8-thiol	Grapefruit, sulfurous, fruity	Orange juice (G), grapefruit juice (D, F, H), grape musts (E)
3-(Methylthio)propanal	Boiled potatoes, baked potato, nutty, stale	Muscadine grape juice (A), grape musts (E)
3-Mercapto hexan-1-ol	Grapefruit	Grapefruit juice (H)
3-Mercapto hexyl acetate	Grapefruit	Grapefruit juice (H)
4-Mercapto-4-methyl-2-pentanol	Grapefruit, sulfury	Grapefruit juice (D, H)
4-Mercapto-4-methylpentan-2-one	Catty, blackcurrant-like	Grapefruit juice (D, H)
bis-(2-methyl-3-furyl)disulfide	Meaty	Grapefruit juice (H)
Methional	Cooked potato	Orange juice (G), grapefruit juice (D, F, H)

[a]References for aroma characterization: A: Baek and others (1997); B: Su and Wiley (1998); C: Bazemore and others (1999); D: Buettner and Schieberle (1999); E: Serot and others (2001); F: Buettner and Schieberle (2001a); G: Buettner and Schieberle (2001b); H: Lin and others (2002); I: Rega and others (2003).

Terpenoid compounds have piney, floral characteristics, while alcohols and aldehydes have green, grassy aromas. In addition, nonvolatile flavor components, including sugars, acids, and bitter compounds are important contributors to the overall flavor quality of juices. Consumer acceptability of these juices is not only dependent on the presence of character-impact compounds, but also the appropriate balance of volatile and non-volatile flavor compounds (Jella and others 1998).

The major categories of juice products include frozen concentrated, pasteurized/refrigerated, refrigerated from concentrate, and aseptically packaged. The flavor of fruit juices is optimal immediately following extraction of the juice. Subsequent processing and storage contributes to alterations in the content and composition of the volatile flavor compounds and the overall flavor quality of the juice. This chapter will provide an overview of the volatile flavor compounds that contribute to the unique flavor of fruit juices and discuss the effects of processing, packaging, and storage on those flavor compounds that are important to the characteristic flavor of fruit juices.

37.2 PRODUCTION OF FLAVOR COMPOUNDS DURING FRUIT MATURATION

The synthesis of volatile and nonvolatile flavor compounds during maturation has a significant impact on the flavor quality of the juice. Enzymatic transamination and decarboxylation reactions of branched-chain amino acids are important in the biosynthesis of

esters, alcohols, aldehydes, and acids which are key contributors to the characteristic flavors of all ripening fruits. Terpenoid compounds, synthesized through the isoprenoid pathways, are important components of citrus flavor. These compounds exist as free and nonvolatile, bound terpenoid glycosides. During fruit maturation, endogenous β-glucosidases catalyze the release of volatile terpineols, including linalool, benzyl alcohol, and 2-phenylethanol, to enhance juice aroma (Gueguen and others 1996).

The degradation of starches to form sugars and the synthesis of acids through the metabolic pathways during ripening contributes to the sweet and tart taste characteristics associated with ripened fruits. Fructose, glucose, and sucrose are the primary sugars found in most fruits. The dominant organic acids present are characteristic of the specific fruit. The sugar to acid ratio, frequently reported as brix/acid ratio, is influenced by many factors, including fruit maturity. The brix/acid ratio has a significant impact on the perceived sweetness and tartness of the juices, quality, and consumer acceptability (Fellers and others 1988).

37.2.1 Citrus Fruit

Terpenes, esters, aldehydes, and alcohols are important constituents in the aroma of citrus juices (Fig. 37.1). The water-soluble compounds, including the esters and monoterpene alcohols, are associated predominantly with the sera of the juice and are extracted from the juice sacs. The monoterpene and sesquiterpene hydrocarbons and other oil-soluble compounds are extracted from the juice and from the peel and are associated predominantly with the pulp and cloud (Brat and others 2003; Moshonas and Shaw 1994).

Neral, geranial, valencene, linalool, β-sinenasal, β-myrcene, α-pinene, and ethyl butanoate are important contributors to orange aroma (Marin and others 1992; Bazemore and others 1999; Buettner and Schieberle 2001b; Rega and others 2003). Limonene, the most abundant compound in orange juice, has a very high odor threshold. Researchers disagree on the contribution of limonene to orange juice aroma. Marin and others (1992) concluded that limonene has a negligible impact on orange juice aroma, while Rega and others (2003) demonstrated that limonene does contribute to overall orange juice aroma. The contribution of specific volatile compounds to flavor depends on the cultivar. Compounds with fruity aroma characteristics, such as ethyl 2-methylbutanoate, ethyl

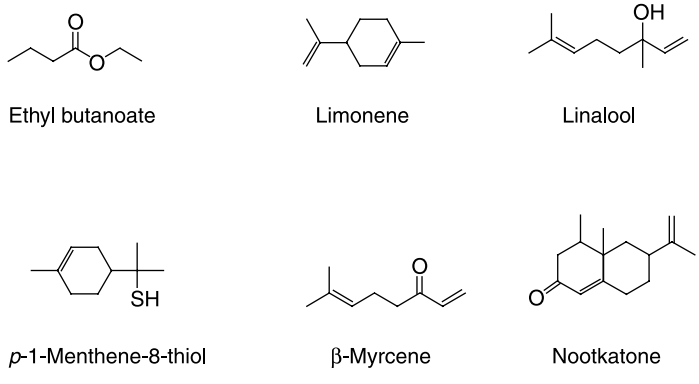


Figure 37.1 *Contributors to aroma of orange and grapefruit juices.*

butanoate, and *cis*-3-hexenal were the most important odor active compounds in Valencia orange juice, while *cis*-3-hexenal, ethyl 2-methylbutanoate, and acetaldehyde were most predominant in Navel orange juice (Buettner and Schieberle 2001b).

Nootkatone, *p*-1-menthene-8-thiol, and ethyl butanoate are character impact compounds of grapefruit (Rouseff and others 1994; Burgard 1995; Shaw and others 1995; Buettner and Scheiberle 1999; Cheetham 2002). Another thiol, 4-mercapto-4-methylpentan-2-one, present in trace amounts, has been shown to be an important contributor to grapefruit juice flavor because of its low threshold (Buettner and Schieberle 2001a). Although acetaldehyde and limonene are the most abundant volatile compounds present in grapefruit juice, these volatiles do not have a significant impact on overall grapefruit juice flavor (Buettner and Schieberle 2001a). Of the volatile flavor compounds identified in grapefruit juice, the contents of myrcene, β-caryophyllene, and linalool have a significant effect on acceptability. Grapefruit juice acceptability was positively correlated with the content of β-caryophyllene, and negatively correlated with the content of myrcene and linalool (Jella and others 1998).

The stereoisomers, geranyl acetate and neryl acetate, and citral, contribute to lemon flavor. Tangerine flavor is attributed to the presence of γ-terpinene, dimethylanthramilate, and α-sinenasal. In addition to these terpene compounds that contribute unique flavor characteristics to citrus juices, esters, aldehydes, and alcohols are also important contributors to the flavor of these citrus juices (Burgard 1995; Cheetham 2002; Rouseff and others 1994; Shaw and others 1995).

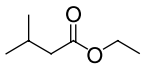
Sugar contents of citrus fruits range from 4% to 7%, depending on the specific fruit and cultivar. Sucrose is the predominant sugar in orange and grapefruit juices and fructose the predominant sugar in lemon juice (Braddock and others 1988). Citric acid, the key organic acid in citrus fruit, contributes sour flavor characteristics. Lemons and limes tend to be high-acid fruits, while oranges, tangerines, and grapefruit tend to be low-acid fruits (Matthews and others 1990; Rouseff and others 1994).

Bitterness in citrus fruits is attributed to limonin, a 22-carbon triterpenoid dilactone, and naringin, a 15-carbon glycosylated flavonoid. Bitterness from naringin is perceived immediately. However, bitterness from limonin is only perceived if the extracted juice is allowed to stand or is heated (Matthews and others 1990; Rouseff and others 1994).

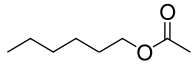
In juices processed from Valencia oranges, early samples were characterized as having more bitter, sour, artificial, and cooked flavor characteristics, with low natural/fresh flavors than juices from more mature fruit. These sensory characteristics were attributed to high contents of peel oil (i.e., octanal, β-myrcene, α-pinene, and limonene) volatiles and moderate contents of essence (i.e., ethyl butanoate, ethyl hexanoate, hexanal, and acetaldehyde) and sesquiterpene (i.e., valencene) volatiles. As the fruit matured, increases in the contents of the essence and sesquiterpene volatiles and brix/acid ratio contributed to increases in sweetness and fresh/natural sensory characteristics and decreases in the bitter/sour and artificial/cooked sensory characteristics (Burgard 1995).

37.2.2 Apples and Pears

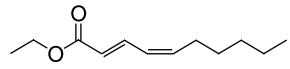
Esters are key contributors to the characteristic fruity aromas of apple and pear juices (Fig. 37.2). Ethyl 2-methylbutanoate, hexyl acetate, ethyl propionate, 2-methybutyl acetate, and butyl acetate are key contributors to apple flavor (Dimick and Hoskin 1983; Young and others 1996; Lopez and others 1998; Cheetham 2002). These esters are noted for their fruity, apple-like aroma (Lopez and others 1998). The composition



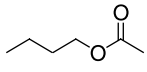
Ethyl 2-methylbutanoate



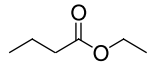
Hexyl acetate



Ethyl *trans*-2-*cis*-4-decadienoate



Butyl acetate



Ethyl butanoate

Figure 37.2 *Contributors to aroma of apple and pear juices.*

and content of the individual volatile flavor compounds in apples has been shown to vary greatly between different cultivars (Poll 1981; Williams and others 1981; Young and others 1996; Lopez and others 1998). The major volatile compound present in pears is ethyl *trans*-2-*cis*-4-decadienoate. Other important volatile flavor compounds include ethyl-2-octenoate, hexyl acetate, ethyl-4-decenoate, butyl acetate, and ethyl butanoate (Cheetham 2002).

The initiation of the climacteric rise in apples and pears contributes to the color, texture, and flavor changes associated with ripening. The release of ethylene contributes to the development of the enzyme systems and volatile precursors necessary for flavor formation. The fatty acid content of the fruit also increases with maturity. Lipid hydrolases, lipoxygenase, and proteases contribute to the formation of the fatty acid and amino acid precursors of flavor formation. In the latter stages of ripening, β-oxidation of linoleic acid, lipoxygenase activity, and esterification of acids with alcohols contribute to ester formation. Since these enzyme systems and flavor precursors are not fully developed in the immature fruit, juice processed from immature fruit often lacks the esters and other volatile compounds that contribute desirable fruity flavor characteristics (Song and Bangerth 1994; Yahia and others 1990).

The storage time and environment of the apples prior to processing into juice also has a significant impact on the flavor of the juice. Apples are frequently held in controlled atmosphere storage to reduce fruit respiration, delay ripening, and extend storage. However, the reduced oxygen environment alters the production of volatile flavor compounds by the fruit resulting in decreases in the contents of butyl and hexyl esters, aldehydes, and ketones and increases in ethanol, acetaldehyde, and ethyl esters (Mattheis and others 1991; Boylston and others 1994; Plotto and others 1999).

In apples and pears, the predominant organic acid and sugar are malic acid and fructose, respectively. In the final stages of ripening, the accumulation of fructose and other sugars is accompanied by decreases in malic acid and starch contents (Blanco and others 1992). The ratio of sugars to acids, which has a major impact on the perceived sweetness and tartness of the juices, varies greatly depending on the specific apple and pear cultivars. Apple cultivars can be classified into four main types: bittersweets, bittersharps, sweets, and sharps. "Bitter" is associated with high polyphenol content, while "sharp" and "sweet" are associated with high and low acid contents, respectively (Dimick and Hoskin 1983).

37.2.3 Grapes

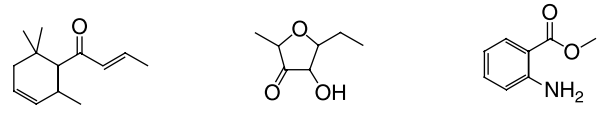
β-damascenone is an important contributor to the flavor of most grape cultivars (Shure and Acree 1994). Other important contributors to the characteristic flavor of grape juices are dependent on the specific grape cultivars. In Concord grapes, odor-active compounds include o-aminoacetophenone, methylfuraneol, and methyl anthranilate (Shure and Acree 1994). In muscadine grape juice, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and o-aminoacetophenone contribute to the characteristic candy and foxy-like aroma characteristics (Baek and others 1997). *p*-1-Menthene-8-thiol and β-damascenone were shown to be important contributors of red fruit-like and grape aroma notes in musts from white grape hybrids (Serot and others 2001). Other contributors to the aroma profile of grape juices include aldehydes and alcohols with green and grass-like aromas, esters with fruity notes, and phenylacetaldehyde and other compounds with sweaty aromas (Baek and others 1997; Serot and others 2001). Significant increases in several important contributors to grape flavor, including o-aminoacetophenone, methylfuraneol, and methyl anthranilate (Fig. 37.3), occur at the onset of the final ripening (Shure and Acree 1994).

Tartaric and malic acids are the predominant organic acids present in grapes. Glucose and fructose are the predominant sugars and are present in relatively equal amounts. The content of the sugars and acids dependent on the grape cultivar and maturity (Buglione and Lozano 2002; Montgomery and others 1982).

37.3 PROCESSING OF FRUIT JUICE

Raw material selection is critical to the quality of the final product. The intensity of the aroma quality of the fruit prior to processing has a significant impact on the overall aroma and taste of the juice. Cultivars and maturity of the fruit have a significant impact on the quality of the final products. Other factors, such as ethylene and respiration rates, climate, soil, and fertilization can also contribute to variations in the volatile flavor compounds in the fruits (Lopez and others 1998). Extraction of the juice from the fruits though pressing is the initial step in juice processing. Depending on the nature of the final product, the extract juice may undergo pasteurization, concentration, and packaging treatments that have a significant impact on the flavor quality of the processed fruit juice.

In orange juice, descriptive sensory evaluation and multivariate statistical analyses showed that juice receiving similar processing treatments clustered into the same category. Fresh-squeezed orange juices were characterized as having raw/fresh and floral sensory notes. Canned juices had strong citrus/non-orange, pungent, sour, bitter, and metallic



β-Damascenone Methyl anthranilate Methyl furaneol

Figure 37.3 *Contributors to aroma of grape juice.*

notes. Frozen concentrated and refrigerated frozen concentrated orange juices were characterized as having orange and candy-like notes (Lotong and others 2003).

37.3.1 Juice Extraction

Juice production involves the pulverization of whole fruit into a puree, followed by pressing to extract the juice from the pomace. Several enzymatic reactions occur during the processing to alter the flavor profile in comparison to the raw materials. Enzymatic cleavage of esters, oxidation of fatty acids to alcohols and aldehydes, and synthesis of alcohols are among the reactions that occur during the processing of juices. Enzymatic oxidation of polyunsaturated fatty acids during the crushing of the fruit contributes to the formation of hexanal, 3-hexenal, 3-hexen-1-ol, and 1-octen-3-one and other oxidation products (Vidrih and Hribar 1999; Serot and others 2001; Williams and others 1981).

Volatile compounds from the peel oil account for 65–80% of the oil-soluble volatile compounds in citrus juices. The peel oil compounds include several terpenoid compounds recognized as important contributors to citrus flavor. The contents of these compounds are significantly higher in mechanically-extracted juice, as compared to hand-extracted juice (Moshonas and Shaw 1994). Separation of the pulp with low speed centrifugation following mechanical extraction results in significant losses of the monoterpene and sesquiterpene hydrocarbons due to their association with the pulp (Brat and others 2003).

37.3.2 Post-Extraction Juice Treatments

The use of styrene divinylbenzene resins for debittering citrus juices reduces limonin and naringin contents by more than 50%. However, the resin also binds the essential oils that contribute to desirable flavor characteristics. The addition of essential oils in commercial processing would compensate for the loss in these desirable flavor compounds (Ifuku and others 1988; Matthews and others 1990).

During apple juice processing, pectinase and other enzyme treatments and microfiltration are performed to increase juice clarity. To optimize these operations, the temperature of the juice is increased to 57°C. At these temperatures, the activity of the native enzymes, such as lipoxygenase, and the release of flavor compounds bound to cell membranes increase the content of several volatile flavor compounds, including isobutyl acetate, ethyl butanoate, ethyl-2-methyl butanoate, hexanal, propyl butanoate, and *trans*-2-hexenal (Su and Wiley 1998).

37.3.3 Thermal and Non-Thermal Processing Treatments

The association of food borne illnesses with the consumption of raw fruit juices has led to a Food and Drug Administration (FDA) ruling that requires juice processors to implement processing treatments that achieve a 5-$\log_{10}$ reduction in the numbers of the most resistant pathogen in the finished product compared to the untreated juice (CFR 2001). The specific processing method to cause a 5-log reduction is not specified by the FDA. Pasteurization, irradiation, and ultraviolet processing are among the treatments shown to be effective in achieving the required 5-$\log_{10}$ reduction. However, these treatments may adversely affect the flavor and other quality attributes of the juice.

Therefore, recent research has focused on the selection of processing treatments that effectively reduce the microbial load without adversely affecting the flavor and other quality attributes of the juice.

37.3.3.1 Pasteurization. As a result of the concerns regarding the safety of juices, 98% of the juice sold is pasteurized as an effective means to destroy all disease-causing pathogens and reduce the number of spoilage microorganisms (Kozempel and others 1998). However, the pasteurization results in significant losses in the natural fruity aromas and the development of cooked aromas due to the thermal degradation of the aroma compounds (Poll and Flink 1983; Poll 1985; Johnson and others 1996; Moshonas and Shaw 1997; Bettini and others 1998; Su and Wiley 1998; Bazemore and others 1999; Wang and others 2003).

Excessive heat treatment of orange juice resulted in the loss of compounds with buttery, fruity-floral, and terpene aroma characteristics and the formation of compounds with vinyl, rotten fruit, cooked grain, and piney aroma characteristics. The changes in aroma profile were associated with the increases in the contents of 4-vinylguaiacol, terpin-4-ol, *cis*-3-hexenal, *trans*-2-nonenal, decanal, and decreases in the contents of ethyl butanoate, propyl butanoate, octanal, methyl octanoate, linalool, and nonanal (Bazemore and others 1999).

In mandarin juices, dimethyl sulfide, furfural, linalool oxide, and 5-methylfurfural were formed during pasteurization. Degradation of S-methylmethionine sulfonium (MMS) during heat processing contributed to the formation of the off-flavor, dimethyl sulfide (DMS). Mandarin juices contain relatively high contents of MMS, although adsorption of MMS by ion-exchange resin effectively reduces the formation of DMS during heat processing and subsequent storage (Ifuku and others 1988).

37.3.3.2 Canning. The severe heat treatment during the canning of orange juice results in significant decreases in flavor quality. The processing results in large decreases in acetaldehyde, ethyl butanoate, and limonene and large increases in α-terpineol. The 10% orange juice drinks are characterized as having poor flavor quality, including the absence of important components for desirable orange juice flavor and high contents of linalool and α-terpineol (Nisperos-Carriedo and Shaw 1990).

37.3.3.3 Irradiation. As a result of concerns of the effects of thermal processing treatments on the flavor quality of juices, researchers have investigated the effects of irradiation treatments on the microbial and flavor quality of fruit juices. Although irradiation does effectively reduce the microbial load in these juices, the flavor quality of the irradiated juices is affected by the irradiation dose and juice environment.

Irradiation of orange juice at 3.5 and 4 kGy resulted in significant changes in the flavor characteristics of the juice. Sensory panelists described the juice as "plastic to decayed" with the increase in irradiation dose resulting in an increased intensity of the off-flavor. Although the irradiation treatment resulted in an increase in the content of total volatile compounds, the effect of irradiation on the characteristic orange juice volatiles and the specific compound responsible for the off-flavor was not determined (Foley and others 2002).

Potassium sorbate, an effective inhibitor of yeasts and molds, is frequently added during the processing of juices to control fermentation and extend the shelf-life

(Baroody and McLellan 1986; Wright and others 2000; Luedtke and Powell 2002). In irradiated apple cider, the presence of sorbate also decreased the rate of loss of esters and other volatile compounds which are important contributors to apple flavor during storage (Crook and Boylston 2004; Crook and others 2004). Sorbate can quench the free radicals that initiate oxidation reactions that form lipid oxidation products and degrade esters and other flavor compounds (Boylston and others 2003).

However, the degradation of sorbate during irradiation has also been shown to contribute to the development of undesirable off-flavors in irradiated juices. Irradiation of orange juice containing sorbic acid was shown to result in the formation of off-flavors at irradiation doses as low as 1.0 kGy (Thakur and Singh 1993). In model systems, descriptive sensory panelists detected a significantly higher intensity of musty off-flavors in irradiated water and apple cider containing sorbate than in either the water or apple cider containing sorbate or the irradiated water or apple cider (Yulianti and others 2005).

37.3.4 Juice Concentration

Concentration of juices, followed by freezing, provides an economical, year-round source for seasonal fruit juices. For these reasons, a large percentage of the juices on the market are reconstituted from juice concentrates. Vacuum concentration, which is often used to produce the concentrates, also results in the removal of volatile flavor compounds. Commercial evaporators use several stages of vacuum-assisted heating to heat the juices to higher temperatures, followed by rapid cooling. During the early stages of the evaporation process for processing juice concentrates, the volatile flavor compounds are vaporized with the water. These volatiles are recovered using an essence recovery system and added back to the frozen concentrates and other processed juice products to enhance the characteristic flavor of the juices (Johnson and others 1996; Sizer and others 1988; Lin and others 2002).

Fresh, pasteurized, and from-concentrate orange juices vary significantly in their flavor quality and consumer acceptability. Processing, either as pasteurization or reconstitution from concentrate, contributes to significant decreases in the contents of volatile compounds, such as ethyl butanoate, ethyl acetate, *cis*-3-hexenol, acetaldehyde, and ethanol and losses in fresh fruit and floral flavor notes in orange juice (Shaw and others 1995). The addition of peel oil to frozen concentrates and reconstituted single strength juices compensates for losses in flavor compounds during processing and results in significantly higher contents of linalool, citral, and limonene than in fresh juice (Nisperos-Carriedo and Shaw 1990).

A comparison of single-strength and reconstituted concentrate grapefruit juice showed that concentration results in more than a 90% loss in volatile compounds. However, not all volatile compounds are lost to the same degree. The fresh/citrusy and sulfur/grapefruit aroma characteristics of the grapefruit juice were almost completely lost during evaporation. This change in aroma profile was attributed to almost complete losses of the three major grapefruit volatile compounds, limonene, caryophyllene, nootkatone, and 30 other volatile flavor compounds. Thermal instability of the flavor compounds and physical evaporation contributed to the losses of these compounds. The aroma compounds with green/fatty/metallic and sweet/fruity characteristics were relatively stable to the effects of thermal evaporation. The contents of ethyl butanoate, 2-*trans*-hexenal, β-ocimene, and linalool oxide were relatively unchanged during processing. *p*-1-Menthene-8-thiol, identified as a key aroma impact compound in grapefruit juice was present only in the concentrated juice and is believed to be a reaction product of hydrogen sulfide with limonene or

α-pinene. Other sulfur-containing compounds, including methional, 2-methyl-3-furanthiol, and bis-(2-methyl-3-furyl)-disulfide, with cooked/meaty aroma characteristics, increased during concentration (Lin and others 2002).

37.4 PACKAGING OF FRUIT JUICES

Single-strength, "not from concentrate" fruit juices packed in glass containers provides the highest quality possible to consumers. However, the high container and shipping costs associated with these products have resulted in interest in application of lighter weight and less expensive packaging material for fruit juices. Plastic packaging is widely used as economic alternatives to glass bottles. The selection of packaging materials has a significant impact on the quality of processed juice during storage. Interactions between foods and packages have a significant effect on the flavor of the food due to adsorption of flavor compounds by the packaging materials, migration of components from the packaging material into the food, and permeation of small gas and water molecules through the packaging material to catalyze chemical degradation of flavor compounds (Sizer and others 1988; Moshonas and Shaw 1989; Letinski and Halek 1992; Marin and others 1992).

37.4.1 Characteristics of Plastic Packaging Materials

The absorption of volatile flavor compounds from juice products by plastic polymers is dependent on the polarity of the volatile compounds. For low-density polyethylene (LDPE), a polymer frequently used in plastic bottles, absorption was inversely related to polarity, with the hydrocarbons, ketones, and aldehydes adsorbed to the greatest degree and only trace amounts of alcohols and esters absorbed by the plastics (Mannheim and others 1987; Sadler and Braddock 1991; Charara and others 1992; Pieper and others 1992; Sadler and others 1995).

Limonene, a terpenoid hydrocarbon present in orange juice, has been shown to be rapidly absorbed by polyethylene packaging materials due to the similarities in the polarity of the volatile compound and packaging material (Mannheim and others 1987; Pieper and others 1992; Sadler and Braddock 1991). Although limonene has not been shown to be a major contributor to orange juice aroma (Marin and others 1992), studies have related losses in orange juice flavor packaged in plastic polymers to absorption of limonene (Kwapong and Hotchkiss 1987). The binding of volatile compounds by LDPE has been shown to increase the oxygen permeability of the packaging material, and thus, contribute to degradation of the volatile flavor compounds (Sadler and Braddock 1990, 1991).

The high absorption of the lipophilic terpene hydrocarbons by LDPE has been attributed to the large amorphous area and low crystallinity of the LDPE polymer. Absorption of volatiles by highly crystalline polymers, such as high-density polyethylene (HDPE), nylon, polyethylene terephthalate (PET), ethylene vinyl alcohol co-polymer (EVOH), and polypropylene (PP), is significantly less than the absorption of volatiles by LDPE (Imai and others 1990; Charara and others 1992; Letinski and Halek 1992; Sadler and others 1995).

A comparison of packaging materials for orange juice flavor showed the greatest losses in volatile flavor compounds occurring for the juices packaged in HDPE and LDPE in comparison to glass or PET. Decreases in the content of the hydrocarbons, limonene, myrcene, and α-pinene, were attributed to adsorption of the volatiles by the polyethylene

packaging material. Oxygen transmission through the polyethylene packages (HDPE and LDPE) contributed to chemical degradation of the aldehydes and esters (Ayhan and others 2001).

Results from sensory evaluation of orange juices packaged in different polymers indicated that the nylon, PET, and EVOH packaging materials did not alter the flavor in comparison to the control treatment. However, the orange juice in contact with LDPE was characterized as having fermented, musty, yeasty, and other spoilage off-flavors. Since D-limonene (or its oxidized or hydrolyzed derivatives) inhibits microbial growth, the reduced content of limonene in the orange juice through absorption by LDPE contributed to higher microbial counts in the orange juice in contact with LDPE in comparison to the other packaging treatments (Sadler and others 1995).

Similarly, sorption of volatile flavor compounds significantly affects the flavor profiles of apple juice. The content of four probe compounds, representative of key apple flavor compounds, was determined during storage in which the apple juice was in contact with LDPE, EVOH, or Co-PET (Co-polyester). The greatest sorption of the volatiles occurred with the LDPE film, in which significant decreases in the contents of ethyl-2-methyl butanoate, hexanal, and *trans*-2-hexenal, but not 1-hexanol, were noted. The EVOH and Co-PET films did not absorb significant amounts of hexanal, *trans*-2-hexenal, or 1-hexanol. Both films did absorb significant amounts of ethyl-2-methyl butanoate, but the level of absorption was lower than for the LDPE films (Konczal and others 1992).

Migration of off-flavor compounds from the plastic to the juice also contributes to losses in flavor quality of fruit juices (Marin and others 1992; Moshonas and Shaw 1989). The LDPE packaging material itself has also been reported to have an off-flavor. Sensory panelists, using a triangle test, were able to distinguish between water samples with and without LDPE strips (Mannheim and others 1987).

The oxygen permeability of the packaging material also has an impact on the flavor characteristics of the juice, especially during storage. Packaging materials with low oxygen permeability, such as Nylon and polystyrene, were more effective than LDPE in the retention of the esters and other volatile flavor compounds that contribute to apple flavor during the storage of apple cider (Crook and Boylston 2004).

37.4.2 Aseptic Packaging

The consumption of fruit juices and 10% fruit juice drinks packaged aseptically in 250 mL flexible, laminated cartons has increased significantly since the 1980s due to the shelf-stable nature of these products. The aseptic carton laminate includes aluminum foil as a barrier in the packaging material and LDPE as the food contact material. Flavor changes during storage of these juice products in laminated cartons limits their shelf-life from one to four months, which is about half that of juices packaged in glass (Mannheim and Havkin 1981; Mannheim and others 1987). The reduced shelf-life of orange and grapefruit juices packaged in laminated cartons was attributed to the absorption of limonene by polyethylene and transmission of oxygen through the packaging material (Mannheim and others 1987).

Deaeration of the juice is a critical step in aseptic processing to remove oxygen that can contribute to degradation of flavor compounds and formation of off-flavors, as well as color and vitamin C. Following pasteurization of the reconstituted juice and the addition

of sterile filtered flavor components the juice filled into the sterilized cartons and hermetically sealed (Sizer and others 1988).

A comparison of reconstituted frozen orange juice to aseptically packed orange juice resulted in the aseptically packed orange juice being significantly less acceptable. A cooked or "prune-like" flavor has been noted in aseptically packed orange juice by descriptive sensory panelists (Moshonas and Shaw 1989).

37.5 STORAGE OF FRUIT JUICES

The shelf-life and flavor quality characteristics of the fruit juices following processing are dependent on the processing and packaging the juice has undergone prior to processing. With the extended storage of juices, losses of volatile flavor compounds contribute to changes in the sensory flavor attributes. In Valencia orange juice, the loss of peel oil (i.e., octanal, β-myrcene, α-pinene, and limonene) and essence (i.e., ethyl butanoate, ethyl hexanoate, hexanal, and acetaldehyde) volatiles resulted in a decrease in the intensity of the fresh/natural flavor note, as evaluated by a sensory panel (Burgard 1995).

Decreased acceptability of aseptically packaged orange juice during a six-week storage period was attributed to decreases in recoverable oil and limonene contents and increases in the contents of the off-flavor compounds, ethyl acetate, and α-terpineol (Moshonas and Shaw 1989). Migration of ethyl acetate from the laminated multilayer carton to the juice contributes to the increase in the content of ethyl acetate (Passy 1983). α-Terpineol is a degradation product of limonene and also contributes to the stale or musty aroma in aged canned orange juice (Tatum and others 1975).

2-Methyl-3-furanthiol, methional, β-damascenone, 5-hydroxymethylfurfural (HMF), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (DMHF), *p*-vinylguaiacol have been shown to increase significantly during pasteurization and storage of orange juice and contribute to storage off-flavors. 2-Methyl-3-furanthiol is formed through the acid-catalyzed degradation of thiamin and has a meaty aroma (Bezman and others 2001; Dreher and others 2003). β-Damascenone, formed through the acid-catalyzed degradation of terpene glycosides, is characterized as rose/tobacco (Bezman and others 2001). Methional, formed from methionine through the Strecker degradation reaction, has a cooked potato aroma (Bezman and others 2001). DMHF and HMF are products of Maillard and sugar degradation reactions (Haleva-Toledo and others 1997). DMHF, at a detection level of 0.1 ppm, has a pineapple-like flavor (Tatum and others 1975). At levels above 0.05 ppm, DMHF masks fresh orange juice aroma (Nagy and others 1989). Nonenzymatic decarboxylation of free ferulic acid results in the formation of *p*-vinylguaiacol, which has an undesirable old fruit or rotten flavor (Naim and others 1988; Tatum and others 1975). The addition of 0.5 mM L-cysteine inhibits the formation of DMHF (Naim and others 1993a) and *p*-vinylguaiacol (Naim and others 1993b) to preserve the fresh aroma of orange juice and minimize off-flavor formation.

During storage, slight changes in content of sugars in grape juice occur through the hydrolysis of sucrose and reaction of the reducing sugars in nonenzymatic browning reactions, with the formation of hydroxymethylfurfural (Cornwall and Wrolstad 1981; Toribio and Lozano 1984; Buglione and Lozano 2002). However, these changes

in sugar profiles most likely have a more significant impact on color and appearance than flavor. Acidity of grape juice is relatively stable during storage (Buglione and Lozano 2002).

37.6 CHALLENGES FOR THE FRUIT JUICE INDUSTRY

Consumption of fruit juices is expected to continue to be a major growth area. The use of juices as carriers of nutrients, making the juices "functional foods" will further contribute to the expansion of the fruit juice market. In addition, the development of juices and juice blends with nontraditional fruits will grow to meet consumers' demands for juices with unique flavor characteristics. With these changes on the horizon, significant opportunities exist for fruit juice processors, food scientists, and flavor chemists to develop processing and packaging technologies to maintain the desirable flavor characteristics of the fruit juices.

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38

Dried Banana

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38.1 Introduction 867
38.2 Drying Methods 868
38.2.1 Sun and Solar Drying 869
38.2.2 Convective Air Drying 870
38.2.3 Microwave Drying 870
38.2.4 Osmotic Dehydration 871
38.2.5 Freeze-Drying 871
38.3 Processing of Some Dried Banana Products 872
38.3.1 Banana Figs 872
38.3.2 Banana Powder 873
38.3.3 Banana Flour 873
38.4 Properties and Nutritional Composition 873
38.5 Packaging and Storing 876
References 876

38.1 INTRODUCTION

Food drying is one of the oldest methods used to preserve food products for later use, being a good alternative to canning and freezing or even a way of complimenting these methods (Andress and Harrison 1999). Drying implies the removal of a significant part of the water from the food, and in low-water-activity conditions, the bacteria, yeasts, and molds cannot grow, and the enzymatic reactions are very slow.

Dried foods are lightweight, take up little space and do not require refrigeration, thus enabling low transportation and storage costs. The dried foods can be consumed (or

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used) in the dry form, or after reconstitution by rehydration. In this operation, water is added back to the product, soaking until the desired volume is restored and the original shape is obtained. In the case of fruits, once reconstituted they are treated as fresh.

Bananas are quite perishable and highly sensible to heat; it is estimated that more than a quarter of the world's production is lost every year. For example, in Papua New Guinea and Ghana the production largely surpasses the needs, but the bad state of the roads does not allow its transportation. However, the banana is also very versatile, thus enabling its consumption in a wide range of products: canned or frozen purée, dried banana figs, banana powder, banana flour, flakes, chips (or crisps), canned slices, jams, and beverages (Dauty 1995). These products can be divided in two categories: those for direct consumption, such as figs, and those for incorporating into other food preparations in the food industry, like purées and powder. Effectively, the majority of the world's production is consumed either raw (in the ripe state) or cooked, and only a small part is processed to obtain a storable product. One of the most important and widespread banana products is the flour prepared by sun-drying of unripe banana and plantain (Dauty 1995).

In South Africa, in Mpumalanga, situated 400 km away from Johannesburg, for 30 years an enterprise has been transforming 10% of its production into dried bananas (in cubes, strips, or slices), which are consumed in the nearby schools. In Uganda, green bananas are sliced and dried to produce "mutere", which is used mainly as a reserve for when there is severe storage of food; it is not, however, a significant part of the local diet under normal conditions (Dauty 1995). Plantains are used in Gabon, dried in slices, for storing or for long journeys, and in Cameroon they are dried in pieces to produce flour, which is used to cook a local recipe known as "fufu". Flour is also produced from dried bananas in some parts of South and Central America and West Indies (Dauty 1995).

Dried bananas make a delicious and inexpensive snack, and, when dried correctly, their nutrients are almost completely preserved. Furthermore, they constitute a good way of increasing the incomes of some communities from a locally available product.

38.2 DRYING METHODS

Bananas, as many other food products, can be dried by conventional drying methods (in the sun, in dehydrators, in an oven, or in an industrial drying chamber) by using a combination of warm temperatures, low humidity, and an air stream to remove the evaporated water (Andress and Harrison 1999).

One of the most common and cheap drying methods, which is practiced in areas of suitable climate, is sun drying. However, ovens or fires are also used to dry bananas by traditional processing techniques. In West Africa, plantains are often soaked and sometimes parboiled before drying. The drying of unripe fruit slices is carried out on bamboo frameworks, cemented areas, mats, swept-bare patches of earth, roofs, or sometimes on stone outcrops or sheets of corrugated iron. In East Africa, peeled bananas are predried on a frame placed over a fire for 24 h, before drying in the sun. In Polynesia, ripe bananas are preserved by wrapping in leaves for storage, after oven drying (Dauty 1995).

Many banana products, such as banana figs or banana flour, are now produced on an industrial scale, using appropriate drying methods. The drying temperature should not exceed 60°C, in order to preserve the product's organoleptic characteristics, and minimize the loss of important nutrients. The drying rate should also be moderate, so as to prevent the product from drying in the outside, impeding the moisture from escaping from the

interior, originating a "case hardening" phenomena. The drying process is accelerated by higher flow rates (by increasing the heat and mass transfer coefficients) as well as by low humidity (by increasing the driving force for moisture transfer).

38.2.1 Sun and Solar Drying

The high carbohydrate content of fruits, and in particular bananas, allows their drying at direct open-air sun exposure. However, this is possible only if the atmospheric conditions are favorable, thus corresponding to sunny, hot, dry and, preferably, windy days. The relative humidity of the atmosphere should be below 60%, and the minimum temperature recommended is 30°C, although higher temperatures are preferable (Andress and Harrison 1999).

For sun drying, the fruits are placed on trays made of screen or wooden dowels. The best screens for a safe contact with the food are those made of stainless steel, teflon-coated with fiber glass or plastic. Screens made from galvanized metal cloth are coated with cadmium or zinc, and therefore must be avoided, because they are subject to oxidation and may leave harmful residues on the food. Also, copper and aluminum screens should be avoided, the first because it destroys vitamin C and increases oxidation, and the second because it tends to discolor and corrode. Most woods are adequate, but some (green wood, pine, cedar, oak, or redwood) are not recommended because they can warp, stain the food, or even cause undesirable flavors (Andress and Harrison 1999).

The trays should be placed on blocks to allow better aeration, and not over the ground because it is moist. If an aluminum sheet is placed under the trays, the temperature is increased by the reflection of the rays on the metal.

To minimize the effect of exposure to insects or birds, the trays can be covered with a cheesecloth, and to prevent rehydration from night moisture, they should be sheltered at night (Andress and Harrison 1999).

To sun dry bananas, firm but ripe fruits are used. After peeling, they are placed (whole or sliced) on the trays and covered with a thin net. The bananas should be turned two or three times a day, for a uniform drying, and the process takes from 3 to 6 days in the case of sliced pieces. The final product is brown, but not hard.

The solar drying methods also use the sun as an energy source, but the drying is not done at direct exposure. For instance, the use of solar dryers has been very promising in drying bananas in Thailand. In the natural convective dryers the products are protected from insects, dust, and rain, and the drying time is reduced when compared to natural sun exposure. However, they present some disadvantages such as relatively low holding capacity and risk of reverse air circulation, causing spoilage of the products. In the forced convection dryers these problems are overcome, but the investments are higher due to the glass-covered solar air heaters required (Schirmer and others 1996).

Schirmer and others (1996) describe a multipurpose solar tunnel dryer that has been developed using a new design concept and has many advantages over conventional solar dryer designs. The dryer comprises a plastic-sheet-covered flat plate collector that supplies energy to heat the air, which is sent to the tunnel by three fans powered by a 53 W solar cell module. This dryer can be used for up to 300 kg of bananas, spread in one layer over a plastic net in the drying tunnel to receive energy both from the incident solar radiation and from the hot air. The temperature of the air in the dryer varies from 40 to 65°C, and the drying time required is about 3–5 days, being approximately half of that required for natural sun drying (5–7 days). Furthermore, the bananas produced in

this dryer have been protected from rain, insects, and dust, and show a superior quality regarding flavor, color, and texture (Schirmer and others 1996).

38.2.2 Convective Air Drying

Brazil is a great banana producer, and the transformation of bananas by drying has been gaining importance over recent years. Dried bananas are produced from different varieties, but all have high sugar contents, are very aromatic, and are nonastringent if dried in the correct ripening state. The fruits are maturated in chambers at temperatures not exceeding 22°C, to prevent the pulp from suffering alterations in texture (being less consistent) or becoming darker and having less aroma, thus disabling their usage (Leite 2004). The maturation chambers should preferably have temperature and relative humidity control, and provide internal circulation and renewal of air. To activate a uniform maturation, different gases are used, such as ethylene or acetylene, the latter being more common because it is cheaper and does not present the risk of explosion in the concentrations used (Leite 2004).

The ripe bananas are washed with water with 50 ppm of chlorine, using a mechanical device, preferably in two stages: first by immersion and then with a water stream. To preserve the natural color of bananas, they are subject to a pretreatment with SO_2, obtained from two different processes. One consists in the burning of sulfur powder in a hermetic chamber, which is a simple and low-cost process, but is difficult to control. The residual value of sulfur in the bananas should be under 100 ppm or 0.01 g/100 g of product (wet basis). Another process is the immersion in an aqueous solution of sodium sulfate, more indicated for the pretreatment of sliced bananas. The 10-mm-thick slices are immersed in a sodium bisulfite solution at 0.4% for approximately 40 s (Leite 2004).

The drying is carried out in a convective air dryer, operated at a constant temperature of 70°C and with an air speed of 2.5 m/s. The trays are loaded at a rate of 14 kg/m^2 in the case of whole fruits or 4 kg/m^2 in the case of sliced bananas. The final moisture content is about 20%, and is reached in 24 h for the whole fruits and 5 h for the slices. In long drying tunnels the temperature can be set to 55°C at the beginning and to 70°C at the end. However, the temperature programs and the speed of the cars can be altered according to each situation (Leite 2004).

Also in Brazil (in Guaraqueçaba), bananas are dried in a convective dryer similar to a tunnel dryer. The fruits are ripened artificially in a chamber, by burning alcohol followed by chamber sealing for approximately 24 h. After this, the room is opened and ventilated for 24–48 h until full ripening of the bananas. When ripe they are selected, with rejection of the fruits not obeying quality standards, and then are peeled manually. The fruits are then placed uniformly over the trays and left in the dryer until they reach a final moisture content of around 25%. The dryer contains four cars with 20 trays of 0.80 × 0.90 m each. The air is heated by six infrared burners, providing a power of 23,012 kJ/h each, and the energy source used is liquid petroleum gas (LPG). The air circulation is provided by an electrical ventilator of 0.6 m diameter, with a motor of 1.47 kW, capable of inducing an air speed of 1.5 m/s. This system allows an important reduction in the drying time, and obtains a final product with clearer color and a more pronounced aroma (Bittencourt and others 2004).

38.2.3 Microwave Drying

Conventional drying methods have a major disadvantage, in the low energy efficiency arising from a constantly decreasing drying rate, which leads to long drying processes.

This is a result of the low conductivity of the foods, which reduces heat transfer inside the product. Therefore, the use of microwaves to process foods, and in particular to dry them, has increased in recent years. This method allows the speeding up of the process, while maintaining the nutritional, medicinal, and sensory properties of the product (Sousa and others 2004a).

The use of microwaves accelerates moisture removal because heat is not transferred from outside the food, but is generated internally by friction of the molecules. Microwave drying equipment also allows a reduction of the physical space required to only 20–35% of that needed for conventional drying (Maskan 2000).

Microwave drying, if not properly applied, may lead to products of poor quality (Yongsawatdigul and Gunasekaran 1996). However, if adequately used, it can produce foods of high quality, with a better aroma and color, with much less energy requirement and processing time when compared to conventional drying (Maskan 2000).

Some research has been done on the microwave drying of bananas (Drouzas and Schubert 1996; Garcia and others 1988; Nijhuis and others 1998; Maskan 2000; Sousa and others 2004a; Sousa and Marsaioli 2004b). The resulting products show an excellent quality and good consumer acceptance, with color, texture, and sweetness close to the accepted ideal (Drouzas and Schubert 1996; Sousa and Marsaioli 2004b). Other authors recommend the combination of conventional convective air drying with microwaves (Prabhanjan and others 1995; Feng and Tang 1998).

38.2.4 Osmotic Dehydration

Osmotic dehydration allows the partial removal of water from fruits by immersion in a hypertonic solution (syrup). The drying force for water removal is due to the difference in the osmotic pressure in the food and in the surrounding solution. The complex cellular structure of the product acts as a semipermeable membrane, allowing the transfer of water from the product into the osmotic solution. The factors that influence the rate of diffusion are the temperature, the osmotic solution concentration, the size and geometry of the product, the mass ratio material/solution, and the level of agitation of the solution (Fernandes and others 2006).

Osmotic dehydration removes water until a certain level is reached, which is still high for preservation purposes, and therefore must be complemented with another drying method, usually by contact with a hot air stream. Fernandes and others (2006) studied the drying of bananas by a combination of osmotic dehydration with convective air drying, and optimized the process by selecting the most appropriate operating conditions that would reduce the total processing time.

Chua and others (2004) used a combination of intermittent infrared and continuous convection heating to dry different samples of banana, which had been osmotically pretreated in sucrose solutions at 15%, 25%, and 35%, to investigate the effects on drying rates and color. They observed that an appropriate control of the intermittences and heat flux intensity regulation, as well as the use of osmotic pretreatments, enables the production of premium quality products while maintaining high drying rates.

38.2.5 Freeze-Drying

Freeze-dried foods are characterized by high-quality characteristics, like superior taste and aroma retention, low bulk density and high porosity, as well as better rehydration

properties, when compared to other drying processes such as air, vacuum, microwave, and osmotic drying (Krokida and others 1998).

Freeze-drying is a dehydration process involving two phases: a first in which the product is frozen, followed by a second in which the water is removed by sublimation. The final product quality depends on the temperature at which the frozen product was kept during the sublimation of ice, and that must be below a critical temperature (the collapse temperature). During freeze-drying, the sublimation of the ice originates pores that may shrink due to surface forces of gravity, thus influencing the porosity of the products. Krokida and others (1998) studied the effect of freeze drying on shrinkage and porosity of some products, and in particular banana, and concluded that for temperatures above the glass transition temperature ($T'_g = -45^\circ C$), the freeze-dried materials shrank, and that an increase in temperature increased bulk density and decreased the final porosity.

38.3 PROCESSING OF SOME DRIED BANANA PRODUCTS

In general, good quality products are obtained from ripe bananas that have, preferably, been harvested green and ripened artificially under controlled conditions at the processing factory.

The primary criteria for accessing maturity is the total sugar content of the fruits, which should be about 19.5%. However, other criteria have been suggested, such as the β-carotene and reducing sugar contents (both of which increase with increasing maturity), as well as pH (which decreases as the fruit ripens). For fruits in the right stage of maturity the β-carotene content should be about 2000 μg/100 g, the reducing sugar content should be less than 1.5%, and the pH should be 5.8 or above. Browning was found to occur if the reducing sugar content was higher than 1.5% (Dauty 1995).

It is important to remove all impurities prior to processing of products, and this is done by washing to remove dirt and spray residues and control on the processing line so that substandard fruit can be removed (Dauty 1995).

After washing, the ripe bananas are peeled, most commonly by hand using stainless-steel knives. However, a mechanical peeler for ripe bananas can also be used, being capable of peeling 450 kg of fruit per hour. For the peeling of unripe bananas and plantains, the fruits are immersed in hot water. For example, immersion for 5 min in water at 70–75°C has been suggested as an aid for peeling green bananas for flour production (Dauty 1995).

38.3.1 Banana Figs

Banana figs, or fingers as they are also known, are usually whole, peeled fruits, carefully dried so as to retain their shape. However, there are some banana figs produced from sliced or halved fruits, which facilitates drying (Dauty 1995).

For the production of banana figs the fruits should be fully ripe, with a minimum sugar content of 19.5% and fully yellow but still firm. The state of maturity of the fruits is very important, because, on the one hand, if they are not mature enough the final product is liable to be tough and lacking in flavor; on the other hand, if overripe fruits are used, the figs tend to be sticky and dark in color (Dauty 1995).

The fruits are peeled and treated with sulfurous acid, and then dried as soon as possible. Various drying systems have been described, where drying takes from 10 to 24 h using temperatures between 50 and 82°C, to obtain products with a moisture content ranging from 8 to 18%. The yield of dried figs is 12–17% of the fresh banana on the stem (Dauty 1995). One factory in Australia uses a solar heat collector on the roof to augment the heat used for drying bananas.

Banana figs can also be obtained by osmotic dehydration, using a technique that involves drawing water from $\frac{1}{4}$ in. thick banana, by placing them in a sugar solution (of 67–70° Brix) for 8–10 h, followed by vacuum-drying (at 65–70°C and a vacuum of 10 mmHg) for 5 h. The moisture content of the final products is 2.5% or less, much lower than that achieved by other methods (Dauty 1995).

38.3.2 Banana Powder

Banana powder is used chiefly in the baking industry for the preparation of fillings for cakes and biscuits and is also used for food preparations for invalids and babies.

In the manufacture of banana powder, fully ripe banana pulp is converted into a paste by passing through a chopper followed by a colloid mill. A 1 or 2% sodium metabisulfite solution is added to improve the color of the final product. Both spray- or drum-drying may be used; however, the latter is more favorable as it allows the recovery of all solids. A typical spray-dryer can produce 70 kg of powder per hour, giving yields of 8–11% of the fresh fruit, but drum-drying gives a final yield of about 13% of the fresh fruit. In the latter method the moisture content is reduced to 8–12% and then further decreased to 2% by drying in a tunnel or cabinet dryer at 60°C (Dauty 1995). Banana powder seems to be able to support storage for up to a year.

38.3.3 Banana Flour

Banana flour is said to be highly digestible and is used in foods for babies and invalids, but can also be used in the preparation of bread and beverages. For the preparation of banana flour, unripe bananas are used. The fruits are harvested at three-quarters the full-ripe stage and are processed within 24 h prior to the onset of ripening. If less mature fruits are used, the flour may taste slightly astringent and bitter due to the tannin content (Dauty 1995).

The production of flour has been carried out by peeling and slicing green fruits, followed by exposure to sulfur dioxide gas. The bananas are then dried in a countercurrent tunnel dryer for 7–8 h, with an inlet temperature of 75°C and outlet temperature of 45°C. After drying, carried out until the bananas reach a final moisture content of 8%, they are milled (Dauty 1995).

38.4 PROPERTIES AND NUTRITIONAL COMPOSITION

The nutritional value of fresh bananas is 92 kcal/100 g of edible portion (Senser and others 1999), and the detailed composition as well as the amounts of vitamins and minerals are listed in Tables 38.1 and 38.2. Bananas have long been recommended as dietary supplements for individuals suffering from digestive disorders due to their anti-ulcerogenic properties. The anti-ulcerogenic agents in banana are various

TABLE 38.1 Nutritional Composition of Bananas.

Component	Composition (g/100 g of Edible Part)[a]
Water	73.9
Protein	1.2
Fat	0.2
Carbohydrates	20.8
Organic acids	0.6
Fiber	2.0
Minerals	0.8

[a] In bananas the edible part is 59% of the fruit (Ferreira and Graça 1977).

Source: Senser and others (1999).

flavonoids, and the major components of this group of polyphenolic compounds are the flavan-3,4-diols (also known as leucoanthocyanidins). However, the anti-ulcerogenic activity is lost if the bananas are submitted to temperatures higher than 50°C (Lewis and others 1999).

The different steps in the processing of bananas, such as preparation, pretreatments, and drying, have an important impact on the final properties of the resulting products.

TABLE 38.2 Amounts of Vitamins and Minerals Present in Bananas.

Component	Composition (per 100 g of Edible Part)
Vitamins	
Carotenes	230 μg
Vitamin B_1 (thiamine)	45 μg
Vitamin B_2 (riboflavin)	55 μg
Vitamin B_5 (pantothenic acid)	230 μg
Vitamin B_6 (pyridoxine, pyridoxal phosphate)	370 μg
Vitamin B_9 (folic acid)	20 μg
Vitamin C (ascorbic acid)	12 mg
Vitamin E (α-tocopherol)	270 μg
Vitamin H (biotin)	6 μg
Vitamin PP (nicotinamide)	650 μg
Minerals	
Calcium	9 mg
Chlorine	110 mg
Copper	130 μg
Fluorine	20 μg
Iodine	3 μg
Iron	550 μg
Magnesium	35 mg
Manganese	530 μg
Phosphorus	30 mg
Potassium	395 mg
Selenium	4 μg
Sodium	1 mg
Zinc	220 μg

Source: Senser and others (1999).

Therefore, the study of changes induced by processing operations assumes an increasing importance for obtaining high-quality products.

The influence of drying temperature on the characterization, sensorial evaluation, and microbial quality of bananas was studied by Leite and others (2006) for bananas obtained in a forced-air drying chamber, under a constant air flow rate of 30 m^3/h at 60 and 70°C. They concluded that the resulting products were in good sanitary conditions, and their chemical composition was not affected by drying. Moreover, the sensorial analysis performed revealed that the products obtained with lower drying temperatures were better accepted.

Boudhrioua and others (2003) have evaluated the changes in aromatic components of banana during ripening and air drying at temperatures of 40, 60, and 80°C. Their results indicated that the changes in aromatic components were dependent on the moisture content of the pulp and on the temperature of the drying air. Furthermore, some components such as the isoamyls strongly decreased during drying, whereas others were partly retained, with elemicine being the most thermal-resistant.

The effect of stepwise change in drying air temperature on the color of dried bananas was studied by Chua and others (2001). They observed that by employing stepwise-varying drying air temperature with appropriate starting temperature and cycle time it was possible to reduce significantly the drying time necessary to reach a desired moisture content, with substantial color improvements.

Demirel and Turhan (2003) investigated the effects of pretreatments and temperature on the final color of dried bananas. The study covered untreated bananas, as well as bananas treated with sodium bisulfite and ascorbic/citric acid, which were dried at temperatures between 40 and 70°C. It was observed that pretreatments and increasing temperature reduced the browning, and that the color change in the untreated bananas was fairly acceptable.

The effect of sugar and NaCl soaking treatments on the quality of dried banana figs was evaluated by Ehabe and others (2006). They concluded that soaking in plain water before drying improved brightness and that the use of a solution of sugar (at 10%, w/v), or NaCl (also at 10%), or even sugar plus NaCl (at 5% each), improved water-binding. Although soaking enhanced the diffusion of solutes into the bananas, their sensory attributes were not significantly altered. However, the use of the composite solution (NaCl + sugar) had some benefits, such as improvement in the sensory qualities as well as in storage capability, by retarding mold development and inhibiting mold growth at the surface.

Sousa and others (2003b) investigated the drying kinetics as well as color and texture changes in bananas that have been processed by osmotic dehydration followed by air-drying. The fruits were submitted for 2 h to sucrose solutions of 45, 55, and 65° Brix and 1 : 2 fruit/syrup ratio at 65°C. Following this pretreatment, the bananas were dried in an oven with air circulation at 65°C. Their conclusions indicated that an increase in the syrup concentration increased browning and texture.

Boudhrioua and others (2002) characterized how the rheological properties and the texture of bananas changed during hot air drying with changes in temperature, as well as the influence of their state of ripeness. The results showed that a radical change occurred after different periods of time (according to the state of ripeness) for drying at a temperature of 80°C. These changes consisted of loss of deformability (becoming brittle) and were due to the product being below the glass transition temperature (T_g) when cooled after drying.

38.5 PACKAGING AND STORING

Banana products are very hydroscopic and highly susceptible to flavor deterioration and discoloration, and, although modern production techniques have developed in this area, the organoleptic characteristics of processed products are still far from those of the fresh fruits. However, this can be overcome to some extent by storing in moisture-proof containers and sulfiting the fruit before drying to inactivate the oxidases (Dauty 1995).

Dried products are also liable to attack by insects and molds if not stored in dry conditions. The sun- or solar-dried fruits can undergo a pasteurization operation prior to conditioning and storage, to kill any insects or eggs that they might contain. In the freezer method, the fruits are sealed in plastic bags appropriate for freezing, which are then placed for 48 h in a freezer at a temperature of $-18^{\circ}C$ or below. In the oven method, the product is placed over trays that are left for 30 min in a preheated oven at around $70^{\circ}C$ (Andress and Harrison 1999). Alternatively, after drying, the products may be disinfested by heating for 1 h to $80^{\circ}C$ or fumigated with methyl bromide or ethylene oxide (Dauty 1995; Leite 2004).

Sousa and others (2003a) studied the stability of two banana products obtained by osmotic dehydration, one at atmospheric pressure and the other under vacuum, complemented by convective air-drying. The bananas were evaluated over 120 days in terms of their sensory, microbiological, and physico-chemical properties. The products were packed in transparent polyethylene terephthalate (PET) containers with a capacity of 200 g, and were stored at room temperature (between 23 and $34^{\circ}C$) and at a relative humidity in the range 33–81%. They concluded that both products were relatively stable in terms of microbial growth and maintained their chemical, physico-chemical, and sensory characteristics. However, the color suffered some significant variation, indicating an increasing tendency for darkening for longer storage periods, due to enzymatic and nonenzymatic browning. This may be explained by the high quantities of sugars present and by the absence of additives for the preservation of color. Finally, they concluded that in terms of acceptance, bananas treated with vacuum were preferable.

The conditioning of bananas can be performed under hermetic conditions, in polyethylene bags of 25 μm thickness containing 10 kg of product. These bags are then placed inside wooden or card boxes and stored in a cool and dry place, protected against external factors such as moisture, insects, and shocks. At this stage no moisture condensation should occur at the surface of the fruit, and the moisture distribution inside the fruit should be uniform. The occurrence of moisture condensation at the surface indicates a moisture content above 23%, making the product unsuitable for commercialization (Leite 2004).

For the commercialization of smaller quantities (200, 250, or 300 g), the fruits are packed manually. The extremities are cut, because they are darker and lower the quality of the product, and then the fruits are weighted and pressed, in a special manual press. Finally, the block obtained is packed inside two layers of plastic film, type PT, the external one being yellow to protect against light. Vacuum packaging is not recommended for fruits like bananas, because the diminution of the pressure inside the package may alter the equilibrium of the water vapor at the surface of the fruit, thus altering its surface moisture, making it sticky and more vulnerable to microbial growth (Leite 2004).

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39

Fresh-Cut Fruits

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39.1 Introduction 879
39.2 Regulatory Issues to be Applied to Fresh-Cut Fruits 880
39.3 Considerations about Raw Materials and their Response to Processing 881
39.4 Processing Conditions 882
39.4.1 Cutting Operations 882
39.4.2 Preserving Techniques for Cut Fruits 884
39.4.2.1 Treatments to Improve Safety 884
39.4.2.2 Treatments to Maintain Sensory Quality 886
39.4.3 Packaging Strategies 888
39.4.3.1 Modified Atmosphere Packaging (MAP) 888
39.4.3.2 Edible Coatings 890
39.5 Guidelines for Commercial Distribution and Storage 891
39.6 Future Prospects 892
References 892

39.1 INTRODUCTION

Fresh-cut fruits and vegetables are among the fastest growing businesses of the fresh produce industry. Fresh-cut commodities are usually offered to the consumer in a state that allows for direct and immediate consumption without need for previous preparation or transformation. Hence, fresh-cut commodities meet the consumers' desire for convenience, without losing fruit freshness and other highly desired quality attributes. However, fresh-cut fruits have lagged in sales behind vegetables because they are more problematic

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in their higher water content and increased physiological activity. Fresh-cut fruit processing is, therefore, still in need of innovative technologies of preservation that allow maintenance of the cut-fruit quality while ensuring safety until consumption.

The term "fresh-cut" refers to any type of processing that physically affects the fruit's original state (trimming, peeling, washing, and/or cutting), but maintains it in a fresh state, near to that of a nonprocessed vegetable tissue. Fresh-cut produce is usually packaged in sealed bags to maintain an adequate environment and is kept at refrigeration temperatures to delay senescence by decreasing physiological respiration, microbial growth, and some quality-related degrading reactions. When fruits are wounded in any way their tissue respiration undergoes a steep rise, causing accelerated consumption of sugars, lipids, and organic acids, and increasing ethylene production, which induces ripening and causes senescence (Kays 1991). In addition, physical damage also triggers a series of decay processes, including oxidative browning, tissue softening, loss of water and nutritive substances, and production of undesirable flavors and odors.

Each fruit may have a different response to fresh-cut processing. This response depends on the extent to which the deteriorative processes occur in a product, which is in turn influenced by several factors such as variety and cultivar, handling during harvest, postharvest, and distribution, and the nature of the processing treatments.

39.2 REGULATORY ISSUES TO BE APPLIED TO FRESH-CUT FRUITS

Specific legislation for fresh-cut products is currently being developed. Most of the existing regulations in both the United States and the EU countries refer only to safety and microbiological quality of the produce. Microbial load limits should be commodity-specific and cut-specific, but, up to now, limits for pathogenic microorganisms in different products have not been established. The French regulations (Ministère de l'Economie des Finances et du Budget 1988) were the first to appear in the EU and are regarded as a reference legislation for many countries. It fixes a maximum contamination value of 5×10^7 cfu/g at the expiration date and bans the presence of pathogenic microorganisms such as Salmonella, whereas *Escherichia coli* or *Listeria monocytogenes* counts are severely restricted.

Improper handling of fresh-cut products may have severe safety risks associated with foodborne illnesses. Implementation of HACCP specific plans for the fresh-cut product industry is not mandatory but strongly advised. The Codex Alimentarius Commission issued a document entitled "Code of Hygienic Practice for Fresh Fruits and Vegetables" (FAO 2003) with an annex for "Ready-to-eat Fresh Pre-cut Fruits and Vegetables" and with the purpose of assisting in the implementation of Good Manufacturing Practices (GMPs), which help to control the microbiological, physical, and chemical hazards associated with the processing of fresh-cut fruits and vegetables. The Annex refers extensively to the Code for fresh fruits and vegetables and to the General Principles of Food Hygiene (FAO 2003) but with special emphasis on some key aspects such as control of food hazards through appropriate facilities, personnel training programs, and adequate documentation and records of the processing and storage stages. A similar document was issued by the U.S. Food and Drug Administration (FDA), "Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce" (FDA 2001), identifying the potential

hazards and reviewing comprehensively the methods of intervention to reduce microbial risks in fresh-cut produce (FDA 2001). A new EU regulation on microbiological criteria for foodstuffs (Official Journal of the European Union 2005) sets a limit of 1×10^2 cfu/g for *E. coli* as indicative of the acceptability of a process. This criterion is set to be applied without prejudice to specific rules affecting other microorganisms, but it is intended to serve as a tool for manufacturers to decide whether a product is ready to be commercialized.

As outlined, information about proper handling of fresh-cut produce is being provided to manufacturers, but efforts should still be strengthened in order to reach consumers and mold their habits with respects to product handling.

The safety of chemicals involved in processing treatments must be carefully considered. Inclusion within the FDA list of GRAS (generally recognized as safe) additives and the Codex Alimentarius list of additives guarantees, in principle, the innocuousness of the treatments applied.

39.3 CONSIDERATIONS ABOUT RAW MATERIALS AND THEIR RESPONSE TO PROCESSING

The state of raw fruits at the moment of processing greatly affects the quality and shelf-life of fresh-cut products. Intrinsic factors such as fruit variety, firmness, size, and ripeness, as well as preharvest, harvest, and postharvest handling are determinants for obtaining high-quality fresh-cut products. Fruit cultivar selection and the ripeness state have a capital importance in most fruits. Different fruit varieties may present different phenolic compositions and amounts of oxidase enzymes, which determine their susceptibility to browning. The stage of maturity may affect both firmness and browning sensitivity. In some fruits, advanced maturity accounts for texture loss and the subsequent solubilization of polyphenol oxidases, leading to an increase in browning sensitivity (Soliva-Fortuny and others 2002b). Ethylene stress response is closely related to fruit maturity. In general, overripe climacteric fruits produce less ethylene but are more sensitive to mechanical damage and microbial alteration (Kader 2002). Ethylene response of partially-ripe fresh-cut apples and pears is double that of ripe fresh-cut fruits (Soliva-Fortuny and others 2002a, 2004a).

Fruit ripeness, together with postharvest handling, also conditions the microbiological quality of the raw produce. Storage under adequate temperature/RH conditions must be conducted in a very few hours after harvest. Sanitation treatments just before processing are advised, especially in fruits with high microbiological risk or in those that have been stored for prolonged periods. Chlorine is extensively used to sanitize whole fruits. Concentrations of up to 1000 ppm have been suggested to sanitize melon and watermelon (Qi and others 1999; Portela and Cantwell 2001). However, chlorine is highly reactive with organic matter, thus losing efficiency and resulting in the formation of potentially harmful substances.

Other sanitizers such as H_2O_2, chlorine dioxide, and organic acids have been used to reduce microbial loads in whole fruits. A treatment with H_2O_2 on whole melon was found to extend the shelf-life of the fresh-cut product by 4–5 days in comparison with chlorine treatment (Sapers and Simmons 1998). The treatment was also effective in decontaminating prunes and table grapes (Sapers 1996) and inactivated *E. coli* in combination with commercial sanitizing agents (Sapers and others 1999). Ukuku and others (2005) achieved total

inactivation of *L. monocytogenes* and at least 4 log cfu/mL reductions of *E. coli* in melon rind rinsed with a washing solution containing 1% H_2O_2 + 25 μg/mL nisin + 1% sodium lactate + 0.5% citric acid. On the other hand, Wisniewsky and others (2000) reported that chlorine dioxide, applied at 16-fold recommended concentrations of 80 μg/mL, could only reduce *E. coli* O157:H7 counts by 2.5 log cfu/mL. Wright and others (2000) found that a 2 min dip in 5% acetic acid was the most effective treatment for inactivating populations of *E. coli* O157:H7 on apple surfaces, achieving three decimal reductions.

Other treatments on whole fruits may be used not only to reduce the microbial loads but also to improve sensory quality maintenance of the processed product. Some studies have assessed the effect that mild heat pretreatments applied to whole fruits have on the quality of the fresh-cut products. Abreu and others (2003) and Steiner and others (2006) reported good results in firmness and color retention of fresh-cut pears and peaches obtained from heat pretreated fruits. UV light can also be used as a pretreatment to improve fresh-cut fruit quality and safety. The microbiocidal effect of UV light is based on its incidence on microbial DNA and to its ability to stimulate resistance mechanisms against pathogens in plant tissues. Yaun and others (2004) achieved a 3.3 log cfu/g reduction of *E. coli* O157:H7 inoculated on whole apples with a UV-C treatment of 24 mW/cm^2. Bai and others (2004) studied the effects of different ethanol vapor, heat, and methylcyclopropene (1-MCP) pretreatments on the quality of fresh-cut apples. Ethanol vapors applied to whole Gala apples at a concentration of 5 mL/kg for 24 h at 25°C and heat treatments of 38°C over 4 d at >98% RH were found to be effective in reducing respiration and wounding ethylene response and prolonging the visual quality of fresh-cut apples at the expense of aroma quality, whereas 1-MCP promoted decay development on the cut surface.

39.4 PROCESSING CONDITIONS

Fresh-cut fruit processing requires the observance of strict handling conditions not only to ensure that microbiological hazards are effectively controlled but also to reduce physiological disorders that are directly related to quality preservation in fresh-cut commodities. Working areas and treatment solutions need to be maintained refrigerated throughout processing in order to reduce the proliferation of microorganisms, the activity of enzymes released due to cutting operations, and the respiratory response of the fruit tissues to mechanical injuries. Microbiological risks in fresh-cut fruits include contamination with human pathogens (FDA 2001). Chilly temperatures do not suffice to stop microbial proliferation. Some foodborne pathogens such as *L. monocytogenes* can even grow under refrigeration (Zagory 1999). Therefore, the implementation of Hazard Analysis Critical Control Points (HACCP) is critical to detect possible risks of contamination. Furthermore, GMPs should be applied to the process to regulate hygiene aspects related to workers' protective clothing (smocks, gloves, caps, and footwear), product handling at the different processing steps, and the cleaning and maintenance operations before, during, and after plant production. Personnel training programs should be designed insisting on the importance of hygiene on quality and safety.

39.4.1 Cutting Operations

Physical damage accounts for most of the detrimental changes that occur in fresh-cut tissues. Cutting and peeling fruits boosts respiration rates and also increases their

susceptibility to microbial spoilage. The removal of the natural protective epidermal barrier and the increase in moisture and dissolved nutrients on the surfaces provide ideal conditions for the colonization and multiplication of microorganisms (Nguyen-The and Carlin 1994). Juice leakage from bruised tissues, containing sugars and organic acids, among other nutritious substances, may allow some fermentative yeasts such as *S. cerevisiae* and *S. exiguous* to grow on fresh-cut produce (Heard 2002). In addition, if sanitation of the whole fruits has not been carried out properly, the risk of cross-contamination with pathogenic microorganisms can be dangerously increased, especially when processing wounded fruits with signs of rot initiation (Garg and others 1990). Hygienization of the whole unprocessed fruits is therefore advised before peeling in order to reduce the initial microbial loads. Furthermore, peeling equipment should allow conscientious cleaning of those components that can be in contact with the product, with special attention to cutting blades. Blades should also be sharpened periodically to maintain cutting performance.

Mechanical damage can be detrimental not only to microbiological stability but also to the appearance and sensory attributes of fresh-cut fruits, which are directly related to consumer acceptability. Tissue browning occurs when the phenolic compounds released from damaged cells are exposed to oxygen and oxidizing enzymes, leading to the formation of dark compounds (Osuga and Whitaker 1995). Phenolic substrates are oxidized to reactive o-quinones that in turn may react with other quinones, phenolic substrates, amino groups of peptides, amino acids or proteins, and other compounds including thiol-containing groups, aromatic amines, or ascorbic acid (Whitaker and Lee 1995). Although browning is mostly influenced by phenolic composition and the amounts of native oxidases, the decompartmentalization and solubilization of these compounds is dependent on mechanical injuries during processing that account for chloroplast breakdown (Rocha and others 1998; Garcia and Barrett 2002).

A common response of fruits to fresh-cut processing includes enhanced respiration and ethylene production and water vapor migration. The increase in respiration rates and water loss promoted by cutting operations affect the structure of cell wall, middle lamella, and cellular membrane, promoting loss of cohesion among cells and resulting in softer and weaker structures. As widely reported for leafy vegetables, turgor pressure has been found to exert a clear effect on the texture of some fresh-cut fruits (Luna-Guzmán and Barrett 2000; Rojas and others 2001). Cell degradation also results in electrolyte leakage and a decay in tissue calcium levels. Therefore, texture loss is strongly determined, among other factors, by the tissue morphology, cellular structure, and composition as well as by the content and delocalization of pectinolytic and proteolytic enzymes (Harker and others 1997). Occurrence of these phenomena may be reduced through the addition of calcium salts. The firmness retention properties of calcium have been attributed to cross linkage of the divalent cation with deesterfied regions of pectins and with cell membranes, imparting improved membrane and cell wall integrity, cell adhesion, and cell turgor (Poovaiah 1988; Picchioni and others 1998). Firmness retention of fresh-cut fruits in response to calcium treatments has been reported on apples and pears (Rosen and Kader 1989; Soliva-Fortuny and others 2002a, 2004), cantaloupe melon (Luna-Guzmán and Barrett 2000), tomatoes (Floros and others 1992), watermelon (Mao and others 2006), and kiwifruit (Agar and others 1999).

The role of ethylene in fresh-cut fruit softening is not yet very well known, but its production in response to wounding does not seem to be inconsequential. Even fruits producing very low levels of ethylene such as melon or watermelon are very sensitive to

ethylene, exhibiting tissue water soaking and overall softening, promoting a loss in soluble solids and some nutritious compounds such as lycopene in watermelon (Mao and others 2006). Other responses induced by ethylene may include enhanced activity of hydrolytic enzymes targeting both cell walls and membranes such as lipoxigenases, polygalacturonases, and phospholipases (Karakurt and Huber 2003), as well as phospholipid catabolism (Mao and others 2004). Some fruit tissues, such as placental tissue in tomato, melon, and watermelon, or core tissue in pome fruits exhibit a significant ethylene production burst in response to fresh-cut processing and therefore should be carefully handled during cutting operations or even removed if necessary.

It is now known that 1-MCP, a potent antagonist to ethylene action, may help to extend the shelf-life of fresh-cut fruits, providing evidence for the role of ethylene in at least some texture deteriorative changes. The action of 1-MCP, generally applied as a pretreatment for the whole nonprocessed fruits, seems to be linked to its ability to bind to the cell membrane of fruits, thus blocking the ethylene binding sites (Laurie 2005). Some research studies have reported promising results in fresh-cut fruits such as apple (Jiang and Joyce 2002; Bai and others 2004; Calderón-López and others 2005), tomato (Jeong and others 2004), pineapple (Budu and Joyce 2003), or watermelon (Mao and others 2006). In general, the beneficial effects of 1-MCP include reduction of weight loss and water soaking, and prolonged firmness. Other reported positive effects in some fruits are a high retention of nutritious compounds such as ascorbic acid and lycopene (Budu and Joyce 2003; Mao and others 2006) or a reduction in the microbial spoilage when combined with calcium dips (Mao and others 2006).

39.4.2 Preserving Techniques for Cut Fruits

39.4.2.1 Treatments to Improve Safety. The naturally occurring microorganisms of most fruits are composed of molds and yeasts, which are in turn responsible for the production of many enzymes participating in the degradative processes that curb their shelf-life. Fungi such as *Botrytis cinerea* and *Aspergillus niger* and yeasts such as *Canidia*, *Cryptococcus*, *Fabospora*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces* have been found to be naturally present on cut fruit surfaces (Chen 2002). Lactic acid bacteria have the ability to grow at low pH and their colonization may happen when low O_2 concentrations are reached in the package headspace just a few days after processing. Lamikanra and others (2000) associated the decay of fresh-cut melon and the accumulation of lactic acid in the product with the proliferation of *acidophilus* bacteria. Most fruits have a low pH that protects them against the growth of pathogenic microorganisms. However, the presence of *Cyclospora cayetanensis*, *Salmonella* spp., and *Shigella* spp. has been reported by Heard (2002) in raspberries, watermelon, and fruit salads, respectively. Dingman (2000) reported the ability of *E. coli* O157:H7 to grow on the moderate pH of wounded apple tissues, which would allow this microorganism to grow on fresh-cut fruits of moderate pH. Kärenlampi and Hänninen (2004) demonstrated that *Campylobacter jejuni* can survive on diced melon and strawberries, whereas *Shigella* spp. has been shown to grow on fresh-cut watermelon and papaya (Fernández-Escartín and others 1989). To reduce the risk of contamination with foodborne pathogens, several antimicrobial treatments can be applied either to transportation and treatment waters, in order to reduce microbial loads through the process, or directly to the product. Washing is one of the first processing operations to which a fruit is subjected. Wash water chlorinated up to 200 ppm is routinely applied to

reduce microbial contamination in produce processing lines. However, the use of chlorine is of concern due to the potential formation of harmful byproducts (Richardson and others 1998).

The use of hydrogen peroxide on fresh-cut fruits has been investigated in recent years. The antimicrobial effect of H_2O_2 lies in its properties as an oxidant. However, the treatment may lead to color changes, depending on the commodity, and residual levels may be variable as a function of the amount of peroxidase in the food system. Park and Beuchat (1999) achieved only <1 log cfu/g reduction in cantaloupe melon treated with a 2% H_2O_2 solution over 2 min. A more intense dipping treatment of 5% H_2O_2 bleached the product surface and had to be discarded. Consistently, Sapers and Simmons (1998) reported reduction of microbial populations on fresh-cut cantaloupe and honeydew melons without modification of their sensory characteristics. Treatment on pome fruits was not advised because problems with H_2O_2 residuals could occur. In addition, the treatment caused anthocyanin-bleaching in red fruits.

Alternative methods for the disinfection of cut surfaces are currently being investigated, some of them based on physical antimicrobial treatments. Ozone has excellent ability to penetrate and does not leave residue. Although it is reactive and may cause physiological injury to the produce, it may be useful for the treatment of process water (Parish and others 2003). Exposure of cut surfaces to UV/visible light can also be a way of reducing microbial loads with minimal effects on the fruit physiology and quality. Lamikanra and Richard (2004) found that UV treatments, applied at desired intensity, induced the formation of copaene, a sesquiterpene phytoalexin with antimicrobial effect that contributed to the fruit response to wounding. In a previous work, UV light also induced formation of phytoalexins in fresh-cut melon, thus improving the fruit defense against microbial spoilage (Lamikanra and others 2002).

Consumer perception of the addition of antimicrobial compounds to minimally processed food is usually negative and it is necessary to explain to them the importance and advantages for certain products. Nevertheless, the demand for products with extended shelf-life still persists. Antimicrobials may tarnish the image that consumers have of fresh-cut fruits and, therefore, the use of compounds obtained from natural sources, such as organic acids, essential oils, or several carbonyl compounds, could be a way of extending the shelf-life of fresh-cut fruits, thus improving the attitude of consumers towards those products containing additives.

Organic acids such as citric are used to preserve fresh-cut fruits not only because they are effective against microorganisms by lowering the pH of the cut surfaces, but also because of the inhibition of some browning reactions. The use of organic acids such as acetic, benzoic, citric, malic, sorbic, succinic, and tartaric, naturally present in plants and plant products, are currently being studied. Treatments with citric acid in the form of lemon juice have been shown to reduce the viability of populations of *S. typhi* and *C. jejuni* inoculated onto cubes of papaya and watermelon, respectively (Fernández-Escartín and others 1989; Castillo and Escartín 1994), with a more pronounced effect on papaya than on watermelon. Carbonyl compounds such as 2-nonanone, a nine-carbon ketone, have been applied in fresh-cut apples to inhibit the growth of *Penicillium expansum* with promising results (Leepipattanawit and others 1997). Some of the natural aroma compounds of fruits and vegetables such as hexanal, hexanol, 2-(E)-hexenal, and 3-(Z)-hexenol, are compounds formed through the lipoxygenase pathway. This enzyme catalyses the oxygenation of unsaturated fatty acids to fatty acid hydroperoxides that are metabolizable to compounds having important roles in plant

defense against microbial growth in wounded tissues (Lanciotti and others 2004). Hexanal can interact with the volatile phase of fruits to form compounds such as hexanol and hexyl acetate, which also act as inhibitors of enzymatic oxidative reactions. Lanciotti and others (1999) observed a strong inhibition of both mesophilic and psychrotrophic microorganisms in apple slices stored under an atmosphere containing 0.15 mmol hexanal/100 g. Consistently, treatments with 150 ppm hexanal or 2-(E)-hexenal and 20 ppm hexyl acetate were lethal on *L. monocytogenes* and prolonged the lag phase period of *E. coli* and *S. enteriditis* (Lanciotti and others 2003).

Some phenolic substances naturally present in fruit tissues such as flavonols and hydroxycinnamic acids have exhibited antioxidant potential and antimicrobial properties (Lule and Xia 2005). Treatments of kiwifruit wedges with low concentrations (1 mM) of cinnamic acid and carvacrol, a major component of the essential oils from oregano and thyme, prevented the appearance of visible spoilage and inhibited growth of the microbial flora for 5 d at 4°C and 8°C (Roller and Seedhar 2002). This treatment prolonged the lag phase period of the microbial flora from less than 1 d in the untreated controls to 3 d at 8°C and 5 d at 4°C. Higher concentrations of the antimicrobials inhibited the onset of microbial spoilage for 21 days, but imparted a pungent and unpleasant aroma to the fruit.

Some plant essential oils may be used to control microbial spoilage in fresh-cut fruits. However, widespread application of these compounds, which are constituted mainly of terpenoids, as natural preservatives is not always possible due to the strong flavors associated with them. Lanciotti and others (2004) reported that citrus, mandarin, cider, lemon and lime essential oils, added at levels compatible with the sensory properties in atmospheres of a fresh sliced fruit mixture of apple, pear, grape, peach and kiwifruit, were able to inhibit the proliferation of naturally occurring microbial populations, to reduce the growth rates of a *Saccharomyces cerevisiae* strain inoculated at levels of 10^2 cfu/g and to increase the death rate of *E. coli* inoculated at levels of 10^6 cfu/mL.

Methyl jasmonate is a plant volatile that acts as an important cellular regulator mediating diverse developmental processes and defense responses. Martínez-Ferrer and Harper (2005) treated diced pineapple with a methyl jasmonate emulsion at a concentration of 10^{-4} M for 5 min. The treatment reduced microbiological loads by 3 log after 12 d storage at 7°C compared with the control pineapple, and did not affect the fruit sensory attributes.

Some microorganisms or their products can also be used as biocontrol agents to limit the growth of pathogenic microorganisms. Leverenz and others (2003) reported that a mixture of lytic bacteriophages in combination with nisin, a broad-spectrum, pore-forming bacteriocin, was capable of reducing *L. monocytogenes* populations by 5.7 log units on melon slices and by 2.3 log units on apple slices.

39.4.2.2 Treatments to Maintain Sensory Quality. Cutting operations, although conducted under controlled conditions, often result into browning, softening, and changes in aroma during storage. To minimize these changes, dips in aqueous solutions containing antibrowning compounds, firming agents, and/or other additives with different purposes can be used. Ascorbic acid (AA) is used in many fruits because of its potential to prevent browning caused by polyphenol oxidase (PPO) enzymes, mostly by reducing quinones to phenolic compounds before they are oxidized to form brown-colored compounds. The AA can also act directly on the enzyme by reducing the cuprous ions of its active site (Osuga and Whitaker 1995). Thiol antioxidants such as glutathione, cysteine, and their derivatives have a great antioxidant potential towards polyphenol oxidases and peroxidases. Three mechanisms have been proposed to explain the

inhibition of enzymatic browning by thiol antioxidants: reduction of quinones back to dihydroxyphenols, direct inhibition by acting on the active site of the enzyme, and formation of a colorless cys-quinone adduct (Gorny and others 2000). Nevertheless, the use of cysteine and thiol antioxidants has some limitations. When cysteine is used as an inhibitor of enzymatic browning on fresh-cut fruits, pinkish-red colored compounds are formed due to phenol regeneration, and the product flavor can be undesirably modified (Richard-Forget and others 1992). Some phenolic compounds that can be naturally found in fruits may also be used to delay browning. 4-Hexylresorcinol is a monophenolic molecule with specific effect on PPO. Organic acids such as citric acid have also been shown to reduce browning in fresh-cut surfaces, in particular combination with a chemical reductant (Sapers 1993).

Combinations of these compounds and their derivatives have been extensively used in the literature to prevent fresh-cut fruit browning (Table 39.1). Most antioxidants may act synergistically in combination with others, in particular when actuating at different levels to stop browning reactions. Some other substances have been found to improve the effect of antioxidants on cut fruit surfaces. Pilizota and Sapers (2004) suppressed core browning of apple wedges treated with an acidic AA + citric acid antibrowning formulation for at least three week storage when using sodium hexametaphosphate as an additive with preservative effect. The addition of calcium chloride may also be effective in delaying browning, probably because calcium has a firming effect on cell membranes that may account for a reduction of the leakage of enzymes and substrates (Sapers and Miller 1992).

The addition of calcium salts to the antioxidant dips may help to improve the texture of fresh-cut fruits during their commercial shelf-life. Calcium cross-links the pectic acid layer found in the cell wall middle lamella (Perera and Baldwin 2001). Several researchers have reported the positive effects of $CaCl_2$ dips to prevent softening in fresh-cut fruits. Luna-Guzmán and others (1999) found that a concentration of 2.5% $CaCl_2$ was optimal

TABLE 39.1 Antioxidant Treatments Recommended to Improve the Visual Appearance of Fresh-Cut Fruits.

Fruit	Dipping Treatment	Reference
Apple	1% AA + 0.5% $CaCl_2$	Soliva-Fortuny and others (2001)
	0.001 M 4-HR + 0.5 M IAA	Buta and others (1999)
	0.75% AA + 0.75% $CaCl_2$	Rocha and others (1998)
	0.01% 4-HR + 0.5% AA	Luo and Barbosa-Cánovas (1997)
	1% AA + 0.2% CA or 0.5% NaCl	Pizzocaro and others (1993)
Pear	2% AA + 1% CaL + 0.5% Cys	Gorny and others (2002)
	1% AA + 0.5% $CaCl_2$	Soliva-Fortuny and others (2002b)
	0.01% 4-HR + 0.5% AA	Dong and others (2000)
	4% NaE + 0.2% $CaCl_2$ + 100 ppm 4-HR	Sapers and Miller (1998)
Peach/nectarine	2% AA + 1% CaL	Gorny and others (1999)
Banana	0.5 M CA + 0.05 M N-Acys	Moline and others (1999)
Melon	2.5% CaL	Luna-Guzmán and Barrett (2000)
Watermelon	2% $CaCl_2$	Mao and others (2005)
Mango	0.001 M 4-HR + 0.5 M IAA	González-Aguilar and others (2000)
Kiwifruit	1% $CaCl_2$ or 2% CaL	Agar and others (1999)
Avocado	1% AA + 0.2% Cys	Dorantes and others (1998)

AA = ascorbic acid, 4-HR = 4-hexylresorcinol, IAA = isoascorbic acid, CA = citric acid, CaL = calcium lactate, Cys = cysteine, NaE = sodium erythorbate, N-ACys = N-Acetylcysteine.

to preserve the texture of fresh-cut melon. However, the use of calcium chloride at such amounts has been reported to impart off-flavors to the cut product, which did not occur in treatments with calcium lactate (Luna-Guzmán and Barrett 2000). Consistently, Manganaris and others (2005) suggested calcium lactate as a potential calcium source for the peach fresh-cut and canning industries. Treatments with calcium lactate improved firmness and visual appearance of fresh-cut peaches without modifying their characteristic flavor and aroma.

39.4.3 Packaging Strategies

39.4.3.1 Modified Atmosphere Packaging (MAP). Modified atmospheres can effectively control the growth of microorganisms on the surface of fresh-cut fruits. Low oxygen atmospheres are especially effective in inhibiting Gram-negative bacteria, but alone are not able to limit the proliferation of Gram-positive microaerophilic species such as *Lactobacillus* (Al-Ati and Hotchkiss 2002). However, some safety concerns have arisen regarding the effects that MAP may have on the naturally occurring microbiota of fresh-cut fruits. Restrictive O_2-atmospheres may inhibit the growth of spoiling microflora, benefiting the proliferation of anaerobic psychrotrophic pathogenic microorganisms such as *L. monocytogenes* (Conway and others 2000; Corbo and others 2005), *E. coli* (Gunes and Hotchkiss 2002), or *Clostridium botulinum* (Larson and Johnson 1999). An appropriate combination of gas composition and package dimensions and permeability adapted to the respiration of the product are critical to reach a sustainable equilibrium of gas concentrations, thus ensuring that O_2 levels inside the packages are high enough to avoid the triggering of anaerobic fermentative processes. Some researchers have studied the effects of superatmospheric levels of O_2 on the microbiological growth on fresh-cut fruits to face the microbiological risk that low O_2 concentrations may pose for consumers. Thus, concentrations ranging from 30 to 100 kPa O_2 have been shown to inhibit the growth of some microorganisms and some oxidative reactions (Kader and Ben-Yehoshua 2000) although levels above 80 kPa seem to be phytotoxic for some vegetable tissues. Results about the effect of high-oxygen atmospheres are, however, often controversial. Van der Steen and others (2003) reported that high-oxygen atmospheres (70 kPa) slightly reduced the maximum specific growth of Gram-negative bacteria but did not affect or stimulated the growth of *L. monocytogenes* and *Aeromonas caviae* (HG4) isolated from fresh-cut produce. These authors also reported that high O_2 concentrations generally inhibited the growth of yeasts, although some particular strains were not affected or even stimulated, which proves that the inhibitory effect is not true for every individual strain.

On the other hand, high CO_2 concentrations are generally effective in controlling the growth of most aerobic microorganisms, especially bacteria and molds, but fail to inhibit most yeasts. In addition, lactic acid bacteria can grow under moderate CO_2 environments (Al-Ati and Hotchkiss 2002). Nevertheless, high CO_2 concentrations may be detrimental to the fruit physiology and, therefore, the package permeability should be enough to evacuate the excess of CO_2 generated by the increase in respiration that occurs after processing.

The effect of high CO_2 levels on respiration and ethylene production appears to be synergistic with low O_2 concentrations. Under these conditions, ethylene wounding response can be severely reduced or almost suppressed, which is an important limiting factor of a great number of physiological reactions in the fruit tissue. Generally, although

CO_2 in small amounts (1%) is necessary for ethylene synthesis, high CO_2 atmospheres have been found to slow down ethylene production. The biosynthetic pathway of ethylene in fruits has been studied for many decades, but the molecular mechanism of how ethylene synthesis is regulated is still not clear. So, the mode of action of CO_2 on this pathway is not yet fully understood, although recent research suggests it has a double effect as, on the one hand, a competitor of ethylene binding and, on the other hand, by reducing the efficiency of the conversion of 1-amino-cyclopropane-1-carboxylic acid (ACC) to ethylene by ACC oxidase. In a similar way, O_2 has been found to be indispensable for the conversion of ACC to ethylene (Yang 1981), which would explain the inhibitory effect of low O_2 concentrations. Most studies on fresh-cut fruits coincide in suggesting storage atmospheres of 1–4 kPa O_2 + 10–20 kPa O_2 for diverse fruits such as melon (Qi and others 1999; Bai and others 2001), watermelon (Cartaxo and others 1997), pear (Gorny and others 2002), or mango (González-Aguilar and others 2000; Poubol and Izumi 2005).

MAP is also used to delay the loss of fresh fruit qualities. However, most MAP conditions alone do not suffice to maintain the visual quality of fresh-cut fruits and their effect on texture is minimal. Packages should be designed to reduce water loss, which may result in texture changes, translucency, and/or surface dehydration, but without facilitating the formation inside the package of water condensates that may favor microbiological proliferation. Passive MAP systems in combination with antioxidant treatments have been suggested to delay browning of fresh-cut apples (Soliva-Fortuny and others 2002a), pears (Senesi and others 1999; Soliva-Fortuny and others 2004a) and mangoes (González- Aguilar and others 2000). Low oxygen concentrations (0.25–5%) in combination with moderate levels of CO_2 (10–20%) have been used to maintain the visual appearance of several fresh-cut fruits such as peach (Palmer-Wright and Kader 1997; Gorny and others 1999), kiwifruit (Agar and others 1999), mango (Rattanapanone and others 2001), and melon (Qi and others 1999; Bai and others 2001). Some studies have assessed alternative MAP concentrations that improve the color retention of fresh-cut fruits. Atmospheres containing 65 kPa N_2O + 25 kPa Ar + 5 kPa O_2 + 5 kPa CO_2 and 90 kPa N_2O + 5 kPa O_2 + 5 kPa CO_2 were shown to be effective in preserving the color of kiwifruit and apple, respectively (Rocculi and others 2004, 2005). Storage under super atmospheric O_2 concentrations has been reported to be effective in inhibiting enzymatic browning in some fresh-cut commodities (Kader and Ben-Yehoshua 2000; Farber and others 2003). Nonetheless, this claim should be corroborated in depth for different fruits.

Research on the effect of MAP on the nutritional composition and bioactive compounds of fresh-cut fruits is still incipient and needs further development. The availability of O_2 in the package headspace as well as high CO_2 levels stimulate the oxidation of ascorbic acid and inhibit the reduction of dehydroascorbic acid (Agar and others 1999; Soliva-Fortuny and others 2003, 2004b). No changes have been reported in the sugar, organic acids, and phenolic contents of fruits as a consequence of MAP (Gil and others 1998; Lamikanra and others 2000; González-Aguilar and others 2000). On the contrary, the aroma profile of fresh-cut fruits can be completely modified by some MAP systems. Gil and others (2002) reported the best overall flavor quality for tomato slices preserved under 2 kPa O_2 + 20 kPa CO_2. Bett and others (2001) reported an increase of the compounds that conform to the flavor of fresh-cut apples a few hours after processing, followed by a dramatic decline that accounted for a loss in product marketability.

39.4.3.2 Edible Coatings. The use of edible materials as a coating has multiple potential applications. Specifically, for fresh-cut fruits, the creation of a modified atmosphere in the fruit and the reduction of water and aroma loss are of special interest. Edible coatings can reduce the gas exchange rates between the fruit product and its environment, thus producing a modified atmosphere. Lee and others (2003) reduced the respiration rate of apple slices with a whey protein concentrate based coating. Wong and others (1994) also reduced respiration and ethylene production of apple slices with a polysaccharide/ lipid bilayer coating. Edible bilayer coatings cast from proteic, lipidic or polysaccharide ingredients are more likely to have improved barrier properties. Polysaccharide and protein polymers are generally of a hydrophilic nature whereas lipidic coatings have better water vapor barrier properties. Le Tien and others (2001) found that a whey protein/ carboxymethyl cellulose coating prevented oxidative browning on apple slices by acting as an oxygen barrier. The addition of a lipid to coating formulations for fresh-cut apples, based on apple purée and pectin, remarkably diminished the gas permeation through the edible matrix (McHugh and Senesi 2000).

TABLE 39.2 Edible Coatings Used on Fresh-Cut Fruits.

Fruit	Coating	Results	Reference
Apple	Chitosan	Delayed microbial growth	Assis and Pessoa (2004)
	Carrageenan/WPC + CMC	Reduced gas transmission; improved sensory properties	Lee and others (2003)
	Beeswax + WPI	Reduced gas transmission browning, and moisture loss	Pérez-Gago and others (2003) McHugh and Senesi (2000)
	Apple purée + pectin + lipid (beeswax, vegetable oils)	Prevented browning due to better gas barrier	Le Tien and others (2001)
	Maltodextrin + MC	Lessened tissue respiration but resulted in surface discoloration	Brancoli and Barbosa-Cánovas (2000)
	Casein + Alginate + AMG	Reduced moisture loss	Pavlath and others (1993)
Pear	MC + Stearic acid	Delayed browning and water loss; high aroma retention	Olivas and others (2003)
Mango	CMC	Prevented discoloration	Nísperos-Carriedo (1994)
Strawberry	Amilose	Gas barrier: improved color and firmness	García and others (1998)
Papaya	Casein + Carnauba wax	Improved gas and moisture barrier properties	Guilbert (1988)
Citrus	Carrageenan + locust bean gum	Reduced water loss	Rouse and Moore (1972)
	Locust bean gum + pectin + grapefruit juice	Reduced water loss and improved overall quality	Bryan (1972)

WPC = whey protein concentrate, CMC = carboxymethyl cellulose, WPI = whey protein isolate, WPC = whey protein concentrate, MC = methylcellulose, AMG = acetylated monoglyceride.

Edible coatings may be used to maintain the aroma and flavor of fresh-cut fruits. Olivas and others (2003) enhanced the aroma retention of pear wedges coated with methylcellulose films with the addition of stearic acid, showing higher production of hexyl acetate and butyl acetate during storage than the uncoated controls. Fatty acids may be used in fruit coating formulations to be incorporated by the fruit into their metabolism as precursors of volatile compounds (Olivas and Barbosa-Cánovas 2005).

Coatings can also act as carriers of antioxidants, antimicrobials, and/or other food additives that may help to control reactions that are detrimental to fruit quality (Baldwin and others 1995). A list of edible films found in the literature to coat fresh-cut fruits is presented in Table 39.2. Baldwin and others (1996) reported that ascorbic acid applied as a component of a coating matrix was more effective in preventing apple browning than when applied by direct immersion of the fruit into an aqueous solution of the antioxidant. The addition of antimicrobial compounds from natural sources to coating formulations has great future prospects in improving the control of microbial growth on the cut fruit surfaces. Chitosan, a polysaccharide produced from chitin found in the exoskeletons of arthropods, has been shown to limit fungal growth in sliced apples (Assis and Pessoa 2004), fresh strawberries (El-Ghaouth and others 1991; Han and others 2005), banana, and mango (Kittur and others 2001).

39.5 GUIDELINES FOR COMMERCIAL DISTRIBUTION AND STORAGE

The commercial shelf-life of fresh-cut fruits can be seriously reduced due to improper handling during storage, distribution, or merchandising. During these stages, temperature is the most important factor to be controlled. Headspace modified atmospheres have multiple benefits for the quality of fresh-cut products but make it very difficult to cool the fresh-cut product once it is packed. Therefore, the product should be cooled before packaging and temperature oscillations should be minimized to avoid condensation phenomena on the cut surfaces and plastic layers. High storage temperatures have a direct impact on quality and safety. Corbo and others (2005) reported inoculated *L. monocytogenes* growing on fresh-cut cactus-pear fruit at any temperature ranging from 4 to 20°C. However, at temperatures greater than 4°C, this proliferation could lead to a significant health risk. *E. coli* O157:H7 was reported to grow in samples stored at 4 and 8°C. Bagamboula and others (2002) found that the growth of *Shigella* spp. on the surface of strawberry and apple fresh fruit salads was also temperature-dependent, claiming that acid products, especially those kept at refrigeration temperatures, may support the survival and growth of *Shigella* spp., causing food poisoning. Low temperatures may also influence the fruit response to deteriorative agents. In fresh-cut melon, low temperatures and reduced oxidative and microbial stress during storage appeared to favor production of cyclic and acyclic phytoalexin terpenoid compounds β-ionone, geranylacetone, and terpinyl acetate, which were effective in inhibiting microbial growth in the fruit (Lamikanra and Richard 2002). On the other hand, Gorny and others (1998b) observed that an increase in the storage temperature from 0 to 10°C reduced by one-half the shelf-life of fresh-cut peach and nectarines due to a rise in respiration and ethylene production. Mass transfer phenomena such as water vapor transfer are also boosted at elevated temperatures. Rivera-López and others (2005) reported weight loss in papaya slices below 5% during 15 days storage at 5°C, whereas this loss was almost trebled at 10°C. Furthermore, storage temperature is the most influential factor affecting the aroma and

composition of fresh-cut fruits. Lamikanra and others (2000, 2002) reported storage temperature as the unique factor influencing the organic acid content in fresh-cut melon and the most significant affecting its volatile profile.

The temperature and return air flow of storage chambers, refrigerated transports, and storage display cases should be adequately controlled and registered in order to ensure maintenance of the cold chain throughout distribution. Most fresh-cut fruits, except for chilling-injury-sensitive commodities like bananas, can safely tolerate storage temperatures of 0°C. The traditional bed of ice used by some retailers to display fresh-cut produce can even be counterproductive. Studies have shown that the food surfaces not touching the ice become warm because of exposure to room temperature. Ice used in refrigeration units may actually act as an insulating layer, hindering product cooling and allowing internal temperatures to increase (IFPA 2006). Information about proper storage and handling conditions should be included in the product labeling so that both retailers and consumers are warned about the impact of high temperatures on quality and the potential safety risks of fresh-cut produce.

39.6 FUTURE PROSPECTS

The fresh-cut fruit industry has a great potential to grow during the next few years, but more research is still needed to find new treatments and to improve those already existing. Innovation in the next years should focus on the following issues:

- To develop new fresh-cut products, obtained from new varieties, by mixing different fruits or with the use of new processing techniques, that are more appealing to consumers;
- To study the effects of different antimicrobial treatments, especially those using compounds obtained from natural sources, on the naturally occurring microflora and foodborne pathogen microorganisms;
- To evaluate the effect of new MAP conditions and edible coatings on the microbiological and physiological stability of fresh-cut fruits;
- To investigate the effects of processing and storage conditions on the nutritional value and health-related compounds of fresh-cut fruits;
- To design specific equipment for the fresh-cut industry that meets hygiene standards, thus allowing processors to control health hazards without affecting the fresh quality of fruits.

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Section XI

Functional Foods

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

40

Functional Foods and Ingredients

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"Let food be thy medicine and medicine be thy food."
– Hippocrates, *c*. 460 – *c*. 377 B.C.

40.1 Introduction 904
40.2 Definition of Functional Foods and Nutraceuticals 906
40.2.1 Functional Foods 906
40.2.2 Nutraceuticals 906
40.3 Effects of Functional Foods and Nutraceuticals on Major Chronic Diseases In Modern Populations 913
40.3.1 Cardiovascular Diseases (CVD) 913
40.3.2 Cancer 913
40.3.3 Obesity 913
40.3.4 Alzheimer's Disease 914
40.4 Sources and Biological Effects of Functional Foods and Nutraceuticals in Nature 914
40.4.1 Flaxseed (*Linum usitatissimum*) 915
40.4.2 Phytoestrogens 915
40.4.3 Tomatoes 916
40.4.4 Garlic (*Allium sativum*) 916
40.4.5 Cruciferous Vegetables 916
40.4.6 Citrus Fruits 917
40.4.7 Cranberries 917

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

40.4.8 Tea 918
40.4.9 Wine and Grapes 918
40.4.10 Chocolate 919
40.4.11 Fish 919
40.4.12 Dairy Products 920
40.4.13 Carbohydrates 920
40.4.14 Beef 921
40.4.15 Vitamins 921
40.4.16 Minerals 921
40.5 Functional Foods and Nutraceuticals: Health Claims and Benefits 922
40.5.1 Health Claims 922
40.5.1.1 Oats 922
40.5.1.2 Psyllium 922
40.5.1.3 Soybeans 922
40.5.1.4 Phytosterols 923
40.5.1.5 Fiber 923
40.5.1.6 D-tagatose 923
40.5.2 Qualified Health Claims 924
40.5.2.1 Selenium and Cancer 924
40.5.2.2 Antioxidant Vitamins and Cancer 924
40.5.2.3 Nuts and Heart Disease 924
40.5.2.4 Omega-3 Fatty Acids and Coronary Heart Disease 924
40.5.2.5 Phosphatidylserine and Cognitive Dysfunction and Dementia 924
40.5.2.6 Folic Acid and Neural Tube Birth Defects 924
40.6 Functional Foods and Nutraceuticals: Safety Issues 924
40.6.1 Echinacea 925
40.6.2 Ephedra 925
40.6.3 Feverfew 925
40.6.4 Garlic 926
40.6.5 Ginger 926
40.6.6 *Gingko Biloba* 926
40.6.7 Ginseng 926
40.6.8 Kava Kava Products 926
40.6.9 St. John's Wort 926
40.7 Regulation of Functional Foods and Nutraceuticals 927
40.8 Public Education and Dietary Guidance 929
40.9 Conclusions 930
References 931

40.1 INTRODUCTION

The terms "functional foods" and "functional food ingredients/nutraceuticals" are applied broadly to foods and food constituents, respectively, that provide specific health or medical benefits, including the prevention and treatment of diseases, as well as nutritional value (the term nutraceuticals is synonymously used for functional food ingredients in several countries). The practice of using certain foods or plants for the prevention or treatment of diseases by human societies must have started far back in time, as hunters and gatherers, and even primates (the closest living relatives of *Homo sapiens*), are known to use medicinal herbs. Hippocrates proclaimed nearly 2500 years ago, "*Let food be thy*

medicine and medicine be thy food." Our ancestors did not know why those plants produced the observed effects, and the discovery of medicinal properties of specific plants and foods was probably accidental or the result of trial and error. It is conceivable that certain edible plants and other foods (but not necessarily the ones that were consumed on a regular basis) were helpful in alleviating sickness, which led to the knowledge of the medicinal properties of such foods. Repeated trial and error could have led to the categorization of specific foods for treating particular diseases or other medical conditions. The use of specific decoctions containing phytochemicals was scientifically practiced in Ayurvedic, Greek, and Oriental systems of medicine, although the precise mechanism of the observed effects of the ingredients was not completely understood.

In modern society, there has been a widespread upsurge of interest in functional foods and functional food ingredients since the mid-1980s. Thus, there has been considerable popular demand for the health-enhancing, physiologically active components of functional foods, as consumers have become increasingly aware of the important link between diet and health and the vital role that diet plays in combating chronic diseases such as cancer and heart diseases.

Functional foods and nutraceuticals include food additives, vitamin and mineral supplements, herbs, phytochemicals, and probiotics. Nutraceuticals can be derived from plant, animal, and microbial sources, including those from the aquatic environment. Phytochemicals are chemical compounds produced by plants such as alkaloids, polyphenols, uncommon amino acids, and so on. Several of these compounds are non-nutritive, but effective in preventing or combating diseases. More than 900 different phytochemicals have been identified as components of different plants. The disease-preventing and disease-combating properties of phytochemicals have been investigated intensively, but further research is needed to enable mankind to benefit from the medicinal properties of as yet unknown plants.

The identification of phytochemicals, together with clinical trials to test their efficacy in the treatment of certain diseases, is essential. It is also necessary to investigate thoroughly the characteristics and biological activity of functional foods and nutraceuticals, such as their therapeutic or disease-preventing efficacy, proper dosage, and possible adverse effects (e.g., interaction with prescription drugs or with other functional foods and nutraceuticals), and so on. At the same time, standardized national and international regulation (e.g., by the United States Food and Drug Administration (FDA), Health Canada, and public health authorities of the European Union) is needed to provide guidelines to ensure the safety and quality of the functional food and nutraceutical products. Important areas of future research in this field include the following:

1. Identification and quantification of promising bioactive components in functional foods;
2. Standardization of bioactive components;
3. Clinical and population studies to assess effects of functional foods and nutraceuticals on human health;
4. Development of standardised analytical methods to enhance and ensure the levels of selected phytochemicals and other biologically active compounds in raw and processed foods;
5. Establishment of proper dosage and delivery systems;
6. Investigation of bioavailability and metabolism of functional foods and nutraceuticals;
7. The study of technical and safety issues that have a bearing on FDA regulation and health claim evaluation (University of Illinois 2000);

8. Examination of regulatory issues;
9. Research on effects of processing on the functional food and nutraceutical products;
10. Stability of the products; and
11. Interaction of functional foods and nutraceuticals with prescription and nonprescription drugs and with other functional foods and nutraceuticals.

40.2 DEFINITION OF FUNCTIONAL FOODS AND NUTRACEUTICALS

40.2.1 Functional Foods

There is, as yet, no universal definition of "functional food". This term was introduced in Japan in the mid-1980s and refers to processed foods containing ingredients that aid specific bodily functions in addition to being nutritious. So far, Japan is the only country that has a specific regulatory approval process for functional foods, which, if approved for public consumption, bear a seal of approval marked "*Foods for Specified Health Use* (FOSHU)" from the Japanese Ministry of Health and Welfare (Arai 1996; Hasler 1998). In the United States and Canada, functional foods are not legally recognized at present; however, many organizations and individuals concerned with the link between foods and health have proposed definitions of this emerging and expanding category of foods. Some examples of such definitions are as follows:

1. "Foods that, by virtue of physiologically active components, provide health benefits beyond basic nutrition" (International Life Sciences Institute);
2. "Any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Institute of Medicine's Food and Nutrition Board) (IOM/NAS 1994); and
3. "A food, either natural or formulated, which will enhance physiological performance or prevent or treat diseases and disorders" (Wildman 2000).

Some examples of functional foods and their physiological effects are listed in Table 40.1.

40.2.2 Nutraceuticals

Nutraceuticals, as defined by Wildman (2000), are naturally occurring substances in foods that have been found to be effective in the prevention or treatment of one or more diseases, or the improvement of physiological performance, thereby enhancing human health. The term functional food ingredients has also been synonymously used for nutraceuticals. Essential nutrients can be considered as nutraceuticals required by the human body. Examples include vitamins C and E, which function as antioxidants.

Health-conscious "baby boomers" have made functional foods a leading trend in the U.S. food industry. In the United States the market value of functional foods is estimated to have been $28.9 billion in 1998 (Waltham 1998) and is expected to be as high as $37.7 billion by 2007. Moreover, the great potential ability of functional foods to mitigate and prevent diseases and promote health and well-being could bring about a significant reduction in overall national health-care costs. The most prominent nutraceuticals in the Western world have included plant fibers, β-carotene, omega-3 (n-3 or ω-3), and polyunsaturated fatty acids, polyphenols, and so on, and the number of known nutraceuticals has been increasing

TABLE 40.1 Examples of Functional Foods.

Functional Foods	Bioactive Components (Known)	Health Benefit or Physiological Effects	Other Sources
Berries (especially blueberry)	Anthocyanins and anthocyanidins	• Neutralize free radicals • May reduce risk of cancer, heart diseases and age-related diseases	Blackberry, raspberry, brightly colored vegetables, eggplant skin, red cabbage
	Catechins	• Neutralize free radicals • May reduce risk of cancer	Tea, grapes
Cantaloupe	β-Carotene	• Fighting cancer	Carrots, pumpkins
Celery	Flavones: apigenin	• Neutralize free radicals • May reduce risk of cancer	Parsley
Citrus fruits	Flavanones, hesperidin, silybin, xanthohumol, limonoids	• Neutralize free radicals • May reduce risk of cancer	Citrus, milk thistle, hops
Fish oil	• Omega-3 fatty acids (necessary for properly maintaining human and animal health)	• May play a role into reducing risk of CVD • Improve mental function (e.g., attention deficit disorder and Alzheimer's disease) • Improve visual function, anti-inflammatory effect	Cold-water fish (e.g., tuna, salmon, trout, halibut, and mackerel) are rich in omega-3 fatty acids because they eat other fish, algae & zooplankton, which are high in DHA. Breast milk (contains DHA).
	• DHA (docosahexaenoic acid)	• A major component of brain and eye tissue	Canola oil, soybean oil, flax seed and some nuts.
	• EPA (eicosapentaenoic acid) ALA (α-linolenic acid)	• Anti-inflammatory properties • Prevent blood clotting (cause of fatal heart attacks) and lowers LDL and total cholesterol	Omega-enriched eggs.
	Conjugated linolenic acid (CLA)	• May improve body immune system • May decrease risk of certain cancers	Cheese, meats

(*Continued*)

TABLE 40.1 *Continued.*

Functional Foods	Bioactive Components (Known)	Health Benefit or Physiological Effects	Other Sources
Flax seed	Phytoestrogens, lignans	• Phytoestrogen, antioxidant properties	Flax seeds, sesame seeds, rye, grain products, vegetables and berries
	Omega-3 fatty acids, phenolic acids, flavonoids, tocopherols	• May reduce risk of hormone-related cancer (breast cancer) by competing with estrogen receptors in cells and against prostate and colon cancers	
	Soluble and insoluble fibre	• Lowers LDL, total cholesterol and triglycerides by blocking uptake of cholesterol and increased excretion of it from body	
Ginkgo	Ginkgo flavone glycosides	• Improve memory and blood flow to brain • May help cure Alzheimer's disease	
Green tea	Epicatechin (EC) Epicatechin gallate (ECG) Epigallocatechin gallate (EGCG)	• Neutralize free radicals • May reduce risk of cancer	
Grapefruit	Flavonols, quercetin, rutin,	• Anticancer and antioxidant	Grapefruits, gingko, grapes, onions
Oats (FDA approved 1st food specific health claim, January 1997)	Insoluble fiber	• May reduce risk of breast or colon cancers	Wheat bran, grain, many vegetables
	Soluble fiber (β-glucan)	• Cholesterol lowering effect (reduces risk of CVD)	Oats, apples, many fruits and vegetables
Olive oil (*Olea europaea*, Oleaceae)	Oleic acid, palmitic acid, linoleic acid, stearic acid	• Increase HDL, anticancer, antioxidant	*Olea europea* (olive)
Onions (yellow and red)	Quercetin	• May reduce heart disease	Tea, apples, cherries, and red wine

Rosemary (*Rosmarinus officinalis*, *Lamiaceae*	Camosol, α-pinene, camphor, cineole	• Antioxidant; • May prevent cholesterol oxidation • Prevent cancer and Alzheimer's disease	Rosemary
Sage (*Salvia officinalis*)	Anti-acetylcholinesterase (AchE), camphor, α-caryophyllene, cineole, limonene	• Antioxidant, estrogenic, and anti-inflammatory properties • Enhance memory (potential treatment for Alzheimer's disease)	Lamiaceae members
Shallots	Fructo-oligosaccharides	• May improve gastrointestinal health	Shallots, onions
Soybean (*Glycine max*. L.) and Sunflower seed (*Helianthus annus*)	Isoflavones: daidzein, genistein, glycitein and their glycosides	• May protect against heart diseases and some cancer • May lower LDL and cholesterol	Soybean product, kudzu root (pueraria lobata ohwi), red clover
	Protein	• 25g/day may reduce heart disease (Health Claim approved by FDA, 1999)	Soybeans, soy protein concentrate, soy protein isolate, soy-containing food
	Soyasaponins A & B	• May help control blood cholesterol, triglycerides and blood sugar. • Contain anticancer enzymes	Soybeans, legumes
	Carotenoids, α-tocopherol (common form of vitamin E), γ-tocopherol (primary source of vitamin E in diet), fatty acids (linoleic and oleic acids)	• Act as antioxidants, protecting fats, blood and other body fluids from free-radical damage	Tomato, orange, spinach
Spinach	Flavonoids ($>$1500 different types), lutein	• Active against age-related macular degeneration	Leafy vegetables, corn, egg yolk

(*Continued*)

TABLE 40.1 *Continued.*

Functional Foods	Bioactive Components (Known)	Health Benefit or Physiological Effects	Other Sources
Sunflower seed *Helianthus annuus* (Compositae)	Vitamins B_1, B_2, B_3, E, Ca, Mg, Mn, P, K, Cu, Re, Se, Zn, complex carbohydrates, fiber, omega-6 fatty acid, protein, unsaturated fat	• Immune-boosting benefits • Antioxidant • Improve skin health • Regulate blood-fat levels • Aid in tissue repair (e.g., treating eczema)	Tomato, guava, rose hip, watermelon, pink grapefruit
Tomato	Lycopene	• Antiprostate cancer • Antioxidant	
Wine, grapes, pomace	Phenolic (e.g. flavonoids, anthocyanins), resveratrol, ellagic acids	• Antioxidant action, blocks cholesterol oxidation (lower LDL, prevent risk of CVD) • Bind to and prevent the absorption of cholesterol	
Yogurt	*Lactobacillus* sp	• Probiotics	Yogurt

progressively. Currently, the number of recognized nutraceutical substances has increased to more than 100, the more popular ones including isoflavones, tocotrienols, organo-sulfur compounds, conjugated linoleic acid (CLA), carotenoids, and flavonoids. Some examples of nutraceuticals and their sources are listed in Table 40.2.

TABLE 40.2 Examples of Vitamins and Functional Food Ingredients (Nutraceuticals) by Food Sources.

	Food Sources	Effects of Health Benefits
	Plants	
Allicin	Garlic, onions, chives, leek	• Antibacterial • Reduce risk of cancer and CVD, thinning blood
Ascorbic acid	Rose hip, fruits, peppers	• Antioxidant (e.g., regenerate oxidized vitamin E) • Reduce risk of cataracts • Reduce cold symptoms (note: not recommended if one has kidney stones or hemochromatosis)
β-Carotene	Carrots, tomato, yellow squash, broccoli, citrus fruits, cantaloupe, pumpkin, sweet potatoes, tomato, paprika, green vegetables	• Antioxidant (not recommended to smokers – incidence of increased lung cancer when administered alone)
Capsaicin	Pepper fruit	• Anti-inflammatory
Catechin	Teas, berries	• Antioxidant
Cellulose (insoluble fiber)	Most of plants (cell wall), whole grain, bran, cabbage family, peas, beans apples, root vegetables	• Holds water, reduces colonic pressure, reduced transit time of digestion • Lowers cholesterol level and CVD
Coenzyme Q-10 (ubiquinone)	Meat, seafoods	• Antioxidant
Cyanidin	Grapes, raspberries, strawberries	• Antioxidant
Flavonoids and anthocyanins	Fruits, many plants, red wine	• Lowering of cholesterol levels, anti-osteoporosis, anti-carcinogenic, antioxidant
Folic acid (vitamin B_9)	Citrus fruits, vegetables, tomato, grains	• Reduce blood level of homocysteine • Decrease CVD
β-Glucan	Brans of oats, barley, wheat	• Lowering blood lipid
Indoles	Cabbage, broccoli, cauliflower, kale, Brussel sprouts	• Antioxidant, anticancer agents
Isoflavones (daidzein, genistein, and glycitein)	Soybeans, other legumes, red clover	• Anti-osteoporosis, anti-cancer, • Reduce stroke, heart disease, and hot flush
Isothiocyanates	Cruciferous vegetables	• Anticancer
Lecithins	Soybeans	• Lower LDL
δ-Limonoide	Citrus fruits	• Anticancer
Lignin	Plants	• Improve vision
Lutein	Spinach, banana, egg yolk, green vegetables	• Antioxidant • Improve vision
Lycopene	Tomato and its products	• Antioxidant • Antiprostate

(*Continued*)

TABLE 40.2 *Continued.*

	Food Sources	Effects of Health Benefits
Resveratrol	Grapes (skins), red wine	• Decrease LDL and increase HDL • Antioxidant
Quercetin	Red grapes, citrus fruit, onion, apple skin, berries, tea, broccoli	• Lowering blood lipid • Anti-inflammatory • Antioxidant
Selenium	Grains, Brazil nuts, seeds, meat, eggs	• Antioxidant
Soy protein	Soybeans	• Lower the LDL
Tocopherols	Soybean oil, olive oil, corn oil, nuts, seeds, wheat germs, peppers	• Anticancer • Antioxidant • Lowering blood lipid (protects cell membrane, prevents lipid oxidation) (note: high doses may interfere with blood clotting)
	Animals	
Calcium	Milk, meat	• Bone protection
Choline	Meat, fenugreek leaves, fava beans	• Possibly to prevent and treat Alzheimer's disease
Conjugated linoleic acid (CLA)	Meat, milk	• Bone protection
Decosahexaenoic acid (DHA) and eico-sapentaenoic acid (EPA)	Fish oil	• Anti-inflammatory • Reduce CVD
Sphingolipids	Most membrane of animal cells	• Building-block components of lipid in tissues
Ubiquinone (coenzyme-Q_{10})	Almost all organisms	• Electron carriers for the electron transport chain in mitochondria
Zinc	Meat, milk, seafood, liver, eggs	• Requires Zn as part of many prosthetic groups of over 80 enzymes
	Microbials (Naidu 2000)	
L. acidophilus (NCFB 1748)	Yogurt, fermented milk	• Probiotics, gastrointestinal health
Bifidobacterium bifidum	Yogurt, sour milk	• Probiotics, disease prevention from harmful bacteria
Bifidobacterium infantis *Bifidobacterium longum*		• Produce both acetic and lactic acid, which reduces intestinal pH, restrict the production of phenols, ammonia, steroid metabolites, bacterial toxin and vasoconstriction-causing amines.
Streptococcus sailvarius sp. (*Thermophilus*)	Yogurt, soured milk	• Probiotics, beneficial role in the ecosystem of the human gastrointestinal tract

40.3 EFFECTS OF FUNCTIONAL FOODS AND NUTRACEUTICALS ON MAJOR CHRONIC DISEASES IN MODERN POPULATIONS

40.3.1 Cardiovascular Diseases (CVD)

Mortality from CVD in the world as a whole is about 51%, which could perhaps be lowered considerably if we had a better understanding of the causes and processes of pathogenesis. Inflammation of the artery lining is the initiating cause for plaque formation in arteries. Recent results of biochemical, epidemiological, and cell culture research suggest that one of the major risk factors for CVD is inadequate intake of foods containing antioxidant micronutrients such as vitamins E and C, β-carotene, and ubiquinone (coenzyme Q10). Increasing intake of fruits and vegetables containing antioxidants is recommended as a protective measure against cardiovascular diseases (Duthie and Brown 1994). Moreover, eating fish at least twice a week can significantly reduce the risk of suffering heart attack or stroke (Brody 2003).

Omega-3 fatty acids are thought to play a particularly important physiological role in the prevention of CVD (Simopoulos 1997; Dewailly and others 2001; Holub 2002; Hu and others 2002;). Decosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in fish oil are considered essential fatty acids, but they can be formed in the body from another omega-3 fatty acid, α-linolenic acid (ALA). ALA is found in various foods, such as flax seed, spinach, and marine animals that eat organisms containing ALA. ALA is included as an ingredient in several foods.

40.3.2 Cancer

Cancer, which is responsible for the second highest mortality rate after CVD in "developed" countries, correlates with environmental factors and dietary habits (Milner 1994). Epidemiological and clinical observations have provided convincing evidence that a good diet can significantly lower the risk of developing cancer. Many phytochemicals and other substances in foods appear to afford protection against cancer (Delaquis and Mazza 1998; Oomah and Mazza 1998; Bruno and Wildman 2000; DiSilvestro 2000; Guthrie and Kurowska 2000). These include fibers, vitamins A, D, and C, vitamins of the B complex, organo-sulfur compounds found in *Allium* plants (i.e., garlic and onions), ellagic acid and other phenols, flavonoids found in fruits and vegetables, and glucosinolates in cruciferous vegetables such as broccoli (components that, on hydrolysis, yield the biologically active derivatives indole-3-carbinol and isothiocyanates) (Jeffery and Jarrell 2000). These multifarious essential and nonessential nutrients apparently modify the carcinogenic process at specific sites, interfering with carcinogenesis. Diet is not the only factor that determines cancer development, but it provides an easy and potentially effective means of lowering the risk. In-depth research is needed to elucidate the processes by which components of the human diet affect the initiation and growth of cancer cells. One of the objectives of future research should be to reveal in which ways nutritional intervention would be appropriate and effective, providing a basis for realistic recommendations regarding dietary regimes (Milner 1994; Arnot 1998).

40.3.3 Obesity

In recent years obesity has been showing an alarming increase in the developed and developing countries among people of all ages (World Health Organization 2000; The House of Commons 2004). This phenomenon can be attributed to a number of causes, notably

1. The change from "traditional foods" (a well-balanced diet) to "fast foods" containing higher concentrations of undesirable ingredients (e.g., trans and saturated fatty acids, and large amounts of sugar);
2. The hectic pace of modern life, which often prompts busy people to eat more convenient but less wholesome foods; and
3. A lack of exercise and a largely sedentary life-style that commonly involves excessive computer usage and TV watching (often accompanied by the munching of fattening snacks).

Obesity increases the incidence of ailments such as heart disease, diabetes, and cancer. The number of people who are classified as obese (i.e., people whose body mass index exceeds 30) has increased by about 2% both in men and women within the last 20 years. Dietary factors of potential importance for energy balance and fat distribution in humans include

- Macronutrients (e.g., carbohydrate, protein, and fat);
- Micronutrients (e.g., thiamin and Zn); and
- Non-nutrients (e.g., dietary fiber, caffeine, capsaicin, and phytoestrogens).

It is important to understand the relationship (whether biochemical or physicochemical) between energy intake, energy expenditure, and the deposition of fat at various anatomical sites. Furthermore, there are great opportunities for research designed to identify or develop functional foods that may help to ameliorate the growing problem of obesity in modern society (Wahlqvist 1994).

40.3.4 Alzheimer's Disease

Consumption of fish containing the omega-3 fatty acid docosahexaenoic acid (DHA) twice a week has been found to reduce the risk of Alzheimer's disease (AD) by 60% (Morris and others 2003). Morris and others (2003) concluded that DHA "had the strongest protective effect against AD" compared to other omega-3 fatty acids. In contrast, eicosapentaenoic acid (EPA), another omega-3 fatty acid, appeared to have no protective effect, and α-linolenic acid (ALA), which is also an omega-3 fatty acid, protected only people with a specific genotype. In short, DHA may play an important part in the prevention of AD.

40.4 SOURCES AND BIOLOGICAL EFFECTS OF FUNCTIONAL FOODS AND NUTRACEUTICALS IN NATURE

Evidence yielded by several epidemiological studies has shown that people consuming diets rich in vegetables and fruits had, on average, a 50% lower incidence of cancer than those who consumed lesser amounts (Block and others 1992; Potter 1992; Hasler 1998; Jeffrey and Jarrell 2000). It appears that the plant-based diets, besides providing basic nutrients, are sources of phytochemicals that reduce the incidence of chronic diseases such as cancer. Many classes of the biologically active phytochemicals had been identified in the mid-1980s (Steinmetz and Potter 1991a,b), and many more of them are still being identified and isolated. Phytochemicals in fruits and vegetables have important

beneficial effects on human health, including disease prevention, the mechanisms of which are gradually coming to be better understood. It must also be borne in mind, however, that health-enhancing products from plants are not necessarily safe for all individuals (see Section 40.6). In addition, the Nutrition Labeling and Education Act of 1990 (NLEA), which requires nutrition labeling for most foods and allows health-beneficial information on food labels, is helping to heighten awareness of functional foods and optimize their benefits (ADA 1995; Howard and Kritchevsky 1997). Examples of functional foods and nutraceuticals and their respective health benefits are listed in Tables 40.1 and 40.2, respectively, and selected ones are discussed in more detail below.

40.4.1 Flaxseed (*Linum usitatissimum*)

Among the major seed oils, flaxseed oil contains the highest level of the omega-3 fatty acid α-linolenic acid (ALA) (57%) (Oomah and Mazza 1998), but research on flaxseed has mainly focused on lignans, a class of phytoestrogens. Flaxseed contains many important functional components, including high concentrations of protein and dietary fiber, lignin, and other phytochemicals with antioxidant activities, such as flavonoids, phenolic acid, and tocopherols (Oomah and Mazza 1998). The two primary mammalian lignans, enterodiol and enterolactone, are formed in the intestinal tract by bacterial action on plant lignan precursors (Setchell and others 1987; Setchell 1995). Flaxseed is the richest source of mammalian lignan precursors (Thompson and others 1991). Owing to the structural similarity of enterodiol and enterolactone to naturally occurring and synthetic estrogens, some scientists have claimed that these compounds exert both weak estrogenic and antiestrogenic effects. Moreover, they may play a role in the prevention of estrogen-dependent cancers such as breast cancer. In experiments on rodents, flaxseed has been shown to decrease tumors of the colon, mammary glands (Thompson 1995), and lungs (Yan and others 1998). Only a few studies have been conducted to evaluate the possible cancer-reducing potential of flaxseed consumption among humans. They have shown that ingestion of 10 g of flaxseed per day may cause several hormonal changes associated with reduced breast cancer risk (Phillips and others 1993). Aldercreutz (1995) found that urinary lignan excretion was significantly lower in postmenopausal breast cancer patients than in controls eating a normal mixed or lacto-vegetarian diet. Flaxseed is also known to reduce total and LDL cholesterol levels as well as platelet aggregation (Bierenbaum and others 1993; Cunnane and others 1993). Flaxseed, however, cannot be digested unless it is powdered. When it is powdered and consumed, α-linolenic acid (ALA) is released, and this essential fatty acid may help to prevent blood clotting, which can cause fatal heart attacks. Because the whole flaxseed contains both soluble and insoluble fiber, it can reduce constipation and diseases of the diverticula.

40.4.2 Phytoestrogens

Phytoestrogenic compounds (e.g., isoflavones, coumestans, and lignans), which are weak estrogens found in plant food, may have anti-estrogenic effects. Some phytoestrogenic compounds, at the levels consumed in the typical U.S. diet, impart a reduced risk of developing endometrial cancer. A recent investigation revealed an inverse correlation between dietary intake of food containing three classes of phytoestrogens (isoflavones, coumestans, and lignans) and the risk of endometrial cancer development (Horn-Ross and others 2003). The relationships were slightly stronger in postmenopausal women than in premenopausal women.

40.4.3 Tomatoes

Tomatoes have been in the spotlight since the mid-1990s because they contain the health-benefiting compound lycopene, a primary carotenoid. Consumption of products containing lycopene has been linked to a reduced risk of cancer development (Giovannucci and others 1995; Goodman and others 1998; Krinsky 1998; Bruno and Wildman 2000), especially cancers of the prostate, breast, digestive tract, cervix, bladder, and skin, and possibly the lung (Clinton 1998). A study of more than 47,000 men revealed that those who consumed tomato products 10 or more times per week had less than half the risk of developing advanced prostate cancer than controls (Giovannucci and others 1995). The mechanism for prevention of cancer by lycopene may be related to the compound's role as an antioxidant. Lycopene is known to be the most efficient quencher of singlet oxygen in biological systems (Di Mascio and others 1989). This antioxidant function of lycopene may also explain the results of a multicenter European study that demonstrated that levels of carotenoids in adipose tissue were inversely related to the risk of myocardial infarction (Kohlmeier and others 1997).

40.4.4 Garlic (*Allium sativum*)

Garlic is one of the most widely used traditional culinary herbs in the world, and it has long been noted for its medicinal virtues. Indeed, it has been called "Russian penicillin" owing to its antibacterial activity. Many medicinal functions of garlic have been documented in the literature (Alder and Holub 1997; Nagpurkar and others 2000). These include antihypertensive, cholesterol-lowering, cancer-chemopreventive, and antibiotic effects (Srivastava and others 1982). The characteristic aroma and taste of garlic is due to an abundance of oil- and water-soluble organosulfur compounds (e.g., allicin), which are probably responsible for the various medicinal effects of garlic. The intact garlic bulb contains an amino acid called alliin, which is converted to allicin by an enzyme called allinase when garlic bulbs are cut or crushed (Block and others 1992; Koch and Lawson 1996). The allicin spontaneously decomposes to numerous sulfur-containing compounds, some of which have been shown to inhibit tumorigenesis in experiments and to reduce cancer risk in humans (You and others 1988; Dorant and others 1993). Indeed, they apparently reduce the incidence of colon cancer by 50% in postmenopausal women (Steinmetz and others 1994). *Allium* vegetables in general, including onions, are believed to offer protection against cancer of the gastrointestinal tract. However, not all epidemiological studies have shown garlic to be effective against carcinogensis (Steinmetz and Potter 1991a,b). This contradiction may reflect differences in the types and quantities of organosulfur compounds present in fresh garlic and garlic products (Lawson and others 1991).

Garlic has also been shown to reduce the incidence of CVD and hypertension and to lower cholesterol levels (Warshafsky and others 1993; Silagy and Neil 1994a,b; Ernst 1997; Isaacsohn and others 1998). It is not clear, at present, which compound or group of compounds in garlic is responsible for the cholesterol-lowering effect.

40.4.5 Cruciferous Vegetables

Cruciferous vegetables include broccoli, cabbage, cauliflower, Brussels sprouts, collard, kale, kohlrabi, turnip, mustards, horseradish, and watercress. The consumption of cruciferous vegetables has been shown to reduce the risk of developing several kinds of cancer (Verhoeven and others 1996). The cruciferous vegetables have high concentrations of

glucosinolates (a group of glycosides), which have been linked to the anticarcinogenic properties of cruciferous vegetables (Verhoeven and others 1997; Delaquis and Mazza 1998). All cruciferous vegetables store glucosinolates in their vacuoles. They are hydrolyzed by the enzyme myrosinase, which is found in plant cells, to a variety of products such as isothiocyanates and indoles. These hydrolysis products appear to have anticancerous effects, which are under investigation. Although natural and synthetic isothiocyanates as a whole have been shown to prevent cancer in animals (Hecht 1995), attention is currently being directed mainly to a particular class of isothiocynates known as sulforaphanes, which can be isolated from broccoli. Sulforaphanes induce enzymes (e.g., quinone reductases) involved in carcinogen detoxification in the human body (Fahey and others 1997). Three-day-old broccoli sprouts contain concentrations of glucuraphanin (the glucosinolate of sulforaphane) that are 10–100 times greater than those of mature plants (Nestle 1998), and hence may be more beneficial.

40.4.6 Citrus Fruits

Citrus fruits include oranges (*citrus sinensis*), tangerines (*citrus reticulata*), lemons (*citrus limon*), and grapefruit (*citrus paradisi*). There are many physiologically active components in citrus fruits, among them citrus flavonoids and limonoids, which comprise two major classes of compounds that have been extensively investigated. A large number of components in citrus products are pharmacologically active, and they have shown to be capable of preventing or alleviating diseases and promoting health, for example because of their anticancer effects (Tillotson and others 1993; Middleton and Kandaswami 1994; Rouseff and Nagy 1994; Girard and Mazza 1998). Some of the principal nutrients in citrus fruits, such as vitamins C and E, folic acid, dietary fiber, and carotenoids, are thought to play a role in preventing or delaying the onset of major degenerative diseases (e.g., cancer, cardiovascular disease, and cataracts) by counteracting oxidative processes. Several "non-nutrients", including limonoids and flavonoids, appear to block and suppress carcinogens (Hasegawa and Miyake 1996) and may be effective against a variety of spontaneous and chemically induced rodent tumors (Crowell 1997; Gould 1997; Ripple and others 1998).

40.4.7 Cranberries

Cranberry juice has been used to treat urinary tract infections, its effectiveness being attributable to the fact that it is rich in benzoic acid, which acidifies the urine (Blatherwick 1914). In addition to benzoic acid, this juice also contains two other phytochemicals, fructose and a nondialysable polymeric compound, which have the ability to inhibit adherence of *Escherichia coli* to uro-epithelial cells (Schmidt and Sobota 1988). The polymer compound was eventually isolated from cranberry and blueberry juices and was found to inhibit adhesins present on the pili of the surfaces of certain pathogenic *E. coli* cells (Ofek and others 1991). A clinical trial in which 150 subjects were allowed to consume 300 mL of juice per day for 6 months showed significant (58%) reduction in urinary tract infection compared to the control group (Avorn and others 1994). Cranberries evidently help to combat not only urinary infection but also several forms of cancer and heart disease.

Furthermore, there is evidence that cranberries also have the potential to protect animals against brain cell damage resulting from strokes, and that cranberry juice has a statistically significant tendency to diminish the occurrence of brain cell death. Stroke

is the third leading cause of death in the United States and the most common cause of disability in adults (Neto 2003). The specific phytochemicals responsible for these protective effects have not yet been characterized but may include anthocyanins, which are strong antioxidants.

40.4.8 Tea

Next to water, tea is one of the most commonly consumed beverages in the world. Tea, especially green tea, is a potent anticancer agent owing to its high concentrations of polyphenolic compounds (AHF 1992; Dreosil and others 1997; Harbowy and Balentine 1997; Mueller–Klieser and others 2002). The polyphenol content of tea leaves can amount to as much as 30% of their total dry weight. Catechins are the principal polyphenols in tea and the most significant ones from the standpoint of medicinal effects (Graham 1992). In green tea there are four major catechins, that is, epicatechin, epicatechin-3-gallate, epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG), which are similar to flavonoids. EGC and EGCG in particular are known to be effective in preventing cancer (Yamamoto and others 1997). Although the occurrence of such effects in humans is inconclusive, it has been found that consumption of large quantities (5–6 cups per day) of green tea is associated with decreased incidence of breast cancer in Japanese women (Kohlmeier and others 1997; Nakashi and others 1998). The tea polyphenols appear, moreover, to block the formation of nonmelanoma skin tumors. Unlike sunblock, which prevents the skin from absorbing harmful ultraviolet (UV) light, tea polyphenols function metabolically after the skin is exposed to excessive sunlight. The compounds found in both green and black teas inhibit a newly discovered chemical pathway involving an enzyme called JNK-2 that appears to play a key role in the development of tumors (Dong 2003). JNK-2 levels increase after the skin is exposed to sunlight, and remain elevated in the skin area exposed to sunlight, increasing the probability that skin cancers will develop. Dong (2003) believes that tea is effective only if large quantities of it are consumed, suggesting that a topical "cream" containing tea polyphenols is likely to be more effective.

Tea appears to be effective against CVD as well as cancer. A study of elderly men in Netherlands (Hertog and others 1993) who consumed tea as the major source of flavonoids (e.g., quercetin, kaempferol, myricetin, apigenin, and luteolin) in their diet showed reduced mortality from CVD in this population. There are other studies demonstrating a link between consumption of tea and reduction in the incidence of CVD, although the evidence is not yet conclusive (Tijburg and others 1997).

40.4.9 Wine and Grapes

Mounting evidence indicates that wine, especially red wine, reduces the incidence of CVD. St. Leger and others (1979) found a strong inverse correlation between wine intake and death from ischemic heart disease in both men and women from 18 countries. In France, in particular, there is a relatively low incidence of CVD despite the prevalence of a diet rich in dairy fat (Renaud and de Lorgeril 1992). This "French paradox" has been explained, in part, by the ability of red wine to increase HDL cholesterol (Das and others 1999). The beneficial effects of red wine are thought to result from high concentrations of polyphenols, which are extracted from grape skins during fermentation and act as antioxidants. The concentrations of phenolic compounds in red grapes were found to range from 260 to 920 mg/kg, and red wines contained 1800 (Cabernet Sauvignon) to 3200 mg/L

(Petite Sirah) of polyphenols (Kanner and others 1994). The tendency of red wine to prevent CVD could be explained by the ability of phenolic compounds to prevent the oxidation of LDL, a critical factor in the process of atherogenesis (Frankel and others 1993a,b). Moreover, moderate consumption of red wine is associated with a decreased risk of age-related macular degeneration (Obisesan and others 1998). Red wine is also a good source of trans-resveratrol, a phytoalexin found in grape skins (Creasy and Coffee 1988). Resveratrol has been shown to have estrogenic properties (Gehm and others 1997), which may help to explain the observed cardiovascular benefits of drinking wine (Jang and others 1997).

Despite the evidence for effectiveness of wine against CVD, a study of over 100,000 adults in North California demonstrated that consumption of red wine involved no significant reduction of coronary risk (Klatsky and others 1997). It should also be pointed out that excessive consumption of alcoholic beverages has been linked to the development of several types of cancer, such as breast cancer (Bowlin and others 1997), as well as cirrhosis of the liver. Those who wish to enjoy the health benefits of wine without undue risk may consider alcohol-free wine, which has been shown to increase total plasma antioxidant capacity, or grape juice, which is effective in inhibiting the oxidation of LDL (Stein and others 1999).

40.4.10 Chocolate

Chocolate contains flavonoids such as (−) epicatechin, which may promote cardiovascular health as a result of direct antioxidant effects or through antithrombotic mechanisms. Dark chocolate brings about an increase in both the total antioxidant capacity and the (−) epicatechin content of blood plasma, but these effects are considerably reduced when milk chocolate is consumed instead, or when dark chocolate is consumed with milk (Serafini and others 2003). This finding indicates that milk may interfere with the absorption of antioxidants from chocolate *in vivo* and may reduce the potential health benefits conferred by consumption of moderate amounts of dark chocolate (Geleijinse and others 1999; Serafini and others 2003).

40.4.11 Fish

Omega-3 (ω-3 or n-3) fatty acids (e.g., DHA, EPA, and ALA) comprise a class of essential polyunsaturated fatty acids obtained primarily from fish oils. DHA and EPA can be formed in the body from another omega-3 fatty acid, ALA, besides being consumed directly with fish oil. ALA is found in plants, such as flaxseed and spinach, as well as fish and other marine animals that eat organisms containing ALA. DHA is one of most important omega-3 fatty acids and is a major component of brain and eye tissues. The omega-3 fatty acids may play an important role in reducing the incidence of CVD, cancer, mental disorders (e.g., attention deficit disorder, bipolar disease, depression, and Alzheimer's disease), and alcoholism (Hibbeln 2003). In addition, omega-3 fatty acids perform many biological functions that can benefit the heart and blood vessels. They can inhibit the synthesis of substances that promote inflammation, reduce the tendency of the blood to form clots, stabilize the electrical activity of heart, lower triglyceride levels, reduce blood pressure moderately, and improve the functioning of artery linings (www.nytimes.com/2003/0729/health/nutrition/29BROD.html). In October 2000, these findings prompted the American Heart Association to recommend the consumption of at least

two servings of fish per week, especially fatty fish such as salmon, sardines, mackerel, herring, lake trout, tuna, halibut, and anchovies. Besides, the results of a study undertaken to investigate possible effects of fish consumption and the intake of DHA and other types of omega-3 fatty acids on Alzheimer's Disease indicate that weekly fish and DHA consumption reduces the risk of Alzheimer's Disease by 60% (Martek Biosciences Corp. 2003). Finally, note that although fish is an important part of the inuit (Eskimo) diet (Krumhout and others 1985; Harris and others 1990; Harris 1997), which contains high levels of fats, this does not increase the incidence of CVD among these people (Bang and Dyerberg 1972).

40.4.12 Dairy Products

Dairy products, such as milk, cheese, and yogurt, are among the best sources of several important vitamins (e.g., vitamin D and riboflavin) and minerals (e.g., calcium and phosphorus) (Jelen and Lutz 1998). Calcium is an essential nutrient, which prevents osteoporosis and possibly colon cancer. The fermented dairy products, such as yogurt, kefir, and sour milk, are categorized as "probiotics", which are defined as "live microbial food supplements, which beneficially affect the host animal by improving its intestinal microbial balance" (Fuller 1994; Jelen and Lutz 1998; Farnworth 2000). Probiotics have attracted considerable attention, primarily because they display anticarcinogenic, hypocholesterolemic, and antagonistic action against intestinal pathogens (Mital and Gang 1995).

Microorganisms have been used traditionally in the fermentation of milk, lactic acid bacteria being of particular interest. In studies of Masai tribesmen in Africa, hypocholesterolemic effects of fermented milk have been discovered (Mann and Spoerry 1974; Mann 1978). The Masai have low levels of serum cholesterol and a low incidence of CHD, in spite of the high proportion of meat in their diet. It was found that they consume large quantities of fermented whole milk daily. Mital and Gang (1995) performed studies pointing to a role of probiotics in the prevention of colon cancer. It may be due to the presence of lactic acid bacteria, which alter the activity of fecal enzymes (e.g., β-glucuronidase, azoreductase, and nitroreductase) that are thought to play a role in the development of colon cancer.

40.4.13 Carbohydrates

Fermentable carbohydrates that serve as nutrient substrates for the good microflora of the gut make up a class of "prebiotics", which have been defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve host health" (Gibson and Robertfroid 1995; Farnworth 2000). These include dietary fibers, nonabsorbable sugars, sugar alcohols, and fruit oligosaccharides (Tomomatsu 1994; Gibson and others 1996). Oligosaccharides consist of short-chain polysaccharides made up of 3–10 simple sugars linked together. They are found in many fruits and vegetables (e.g., bananas, garlic, onions, milk, honey, artichokes, and chicory). The category of prebiotics has been extended to include "symbiotics", which are mixtures of probiotics and prebiotics (Gibson and Robertfroid 1995). Many symbiotic products are available in the European market.

40.4.14 Beef

A conjugated linoleic acid (CLA) known as an "anticarcinogenic fatty acid" was first isolated from grilled beef (Ha and others 1987). This CLA is an isomer of linoleic acid (18 : 2, n-6), in which the double bonds are conjugated instead of being in the typical methylene-interrupted configuration. Reportedly, there are nine different isomers of CLA that occur naturally in foods. They have been shown to be effective in suppressing stomach tumors in mice and mammary carcinogenesis in rats (Ip and Scimeca 1997). It has even been suggested that they play a role in reducing weight (Park and others 1997).

The highest concentrations of CLAs are found in the fatty fractions of foods obtained from ruminants, for instance, beef, dairy products, and lamb. Beef fat contains 3.1–8.5 mg CLA/g, the 9-cis and 11-trans isomers contributing 57–85% of total CLAs (Decker 1995). A significant increase in the CLA content of meat may result from cooking or processing; yet the obvious advantage of this from the standpoint of cancer prevention is likely to be at least partially offset by the fact that the concentrations of many mutagens and carcinogens increase as well.

40.4.15 Vitamins

Vitamins are defined as biochemical compounds required by the body in small amounts. There are two types of vitamins, water-soluble and fat-soluble. Fat-soluble vitamins, which include vitamins A, D, E, and K, are found mainly in fatty foods such as animal fats (including butter and lard), vegetable oils, dairy foods, liver, and oily fish. They are essential for proper functioning of the body. Excess amounts of vitamins derived from the foods are stored in the liver and fatty tissues for future use, but consumption of an excess can be very harmful. Water-soluble vitamins, which include vitamins B_6, B_{12}, and C, biotin, folic acid, niacin, pantothenic acid, riboflavin, and thiamin (Padh 1994), are found in fruits, vegetables, and grains. As they are not stored in the body, these vitamins must be constantly supplied from dietary sources. In contrast to the fat-soluble vitamins, consumption of larger than essential quantities of water-soluble vitamins is generally not harmful, as the excess amounts are excreted in urine. As a rule, they are unstable when exposed to heat or air. When food is boiled in water, any water-soluble vitamins can be leached into the water and lost. Foods containing water-soluble vitamins are best cooked by steaming or heating in a microwave oven to ensure retention of vitamins.

40.4.16 Minerals

Minerals are essential inorganic nutrients that the human body needs in small quantities and that are often absorbed from food. Minerals are present in varying concentrations in a variety of foods, such as meat, cereals, fish, milk and dairy foods, vegetables, fruits (including dried fruits), and nuts. Minerals such as calcium and phosphorous are necessary for building strong bones and teeth, and sodium and potassium control the level of water inside and outside cells. Many minerals, including magnesium, iron, phosphorus, potassium, sodium, and sulfur, are enzyme co-factors and control the rate of enzyme reactions and energy production within the body (Anderson and Allen 1994).

"Trace elements", such as boron, cobalt, copper, chromium, fluoride, iodine, manganese, molybdenum, selenium, silicon, and zinc, are essential minerals that are needed in minute quantities compared with other minerals (Anderson and Allen 1994). They are found in a variety of foods such as meat, fish, cereal, milk and dairy foods, vegetables, and nuts.

40.5 FUNCTIONAL FOODS AND NUTRACEUTICALS: HEALTH CLAIMS AND BENEFITS

40.5.1 Health Claims

40.5.1.1 Oats. There is evidence that oat β-glucan, like many other polysaccharides, may reduce total and low-density lipoprotein (LDL) cholesterol, thereby reducing the risk of coronary heart disease (CHD) (Wood and Beer 1998). Oats contain β-glucan, which is one of the dietary sources of cholesterol-lowering soluble fiber and has been studied extensively. In support of a petition of health claim, the Quaker Oats company (Chicago, Illinois) conducted clinical trials on the effects of oat consumption by humans between 1980 and 1995. This study revealed that a statistically significant reduction in total cholesterol and LDL cholesterol was achieved in hypercholesterolemic subjects by daily consumption of 34–123 g of oat bran or oatmeal. It also showed that 3 g of β-glucan is required to obtain a 5% reduction of serum cholesterol, meaning that approximately 60 g of oatmeal or 40 g of oat bran had to be consumed. In order to qualify for a health claim, an oat product must contain 13 g of oat bran or 20 g of oatmeal, and provide at least 1.0 g of β-glucan per serving without any fortification (Hasler 1998). On the basis of these studies, the Food and Drug Administration (FDA) awarded the first food-specific health claim in January 1997 (DHHS/FDA 1997) for oats and oat products.

40.5.1.2 Psyllium. FDA extended the soluble fiber health claim to psyllium fiber in February 1998. Psyllium husk, which is marketed as Metamucil and used as an additive in several other products, such as cereals, supplements soluble fiber in the diet. It confers several health benefits, notably alleviation of constipation and a moderate decrease in blood pressure.

40.5.1.3 Soybeans. Besides being a source of high-quality protein, soybean lowers cholesterol levels and reduces the risk of CVD. Soybeans and soy foods contain many phytochemicals that are considered to be effective in preventing and ameliorating many chronic diseases such as cancer, CVD, and osteoporosis, and it alleviates menopausal symptoms (Messina and others 1997). The phytochemicals in soybeans include phytoestrogens (e.g., genistein, daidzein and glycitein, and their glucosides), phytosterols, tocophenols, saponins, phenolic acids, lecithins, protease inhibitors, and phytic acid. The cholesterol-lowering effect of soybean has been well documented. A meta-analysis (1995) of 38 separate studies involving 743 subjects revealed that consumption of soy protein (25 g/day) resulted in a significant reduction in total cholesterol (9.3%), LDL cholesterol (12.9%), and triglycerides (10.5%), with a small increase in high-density lipoprotein (HDL) cholesterol (2.4%) (Anderson and others 1995). However, isoflavones in soy protein did not seem to have any cholesterol-lowering effect (Nestle and others 1997; Hodgson and others 1998). The exact mechanism by which soy protein exerts its

hypocholesterolemic effect has not yet been fully elucidated. Recently, a meta-analysis of the relationship between soy isoflavones and LDL- and HDL-cholesterol concentrations in humans showed decreased LDL cholesterol and increased HDL cholesterol during isoflavone consumption (Weggemans and Trautwein 2003). However, there was no dose–response relation between soy isoflavones and changes in LDL or HDL cholesterol levels, even though the study was carried out by feeding the subjects 36 g of soy protein per day together with 52 mg of soy isoflavones on average. In November 1999, FDA approved a health claim for soy protein on the grounds that daily consumption of at least 25 g of soy protein tends to reduce the risk of CVD.

Among the soybean phytochemicals that have anticarcinogenic effects, isoflavones (phytoestrogens) are the most noteworthy, because soybeans are the only known dietary source of significant amounts of these compounds (Messina and Barnes 1991; Hendrick and Murphy 2000). There are 12 forms of isoflavonoid components. These include three aglycones (daidzin, genistin, and glycitin), three glucosides of aglycones (daidzin, genistin, and glycitin), three acetyl ester glucosides (acetyl-daidzin, acetyl-genistin, and acetyl-glycitin), and three malonyl ester glucosides (malonyl-daidzin, malonyl-genistin, and malonyl glycitin) (Jackson and others 2002). Isoflavones are heterocyclic flavonoid components structurally similar to estrogenic steroids. Owing to their weak estrogenic activity, isoflavones may act as anti-estrogens by competing with the more potent, naturally occurring endogenous estrogens (e.g., 17β-estradiol) for the estrogen receptor. This might be the reason why populations that consume large quantities of soy products daily (e.g., inhabitants of Far Eastern countries) generally have a reduced risk of estrogen-dependent cancers (Messina and others 1997).

Soybean and soy foods may also benefit bone health (Anderson and Garner 1998; Messina and others 2000). A clinical study of 66 postmenopausal women conducted at the University of Illinois (Erdman and Potter 1997) demonstrated that consumption of 40 g of isolated soy protein (ISP) containing 90 mg total isoflavones per day increased both bone mineral content and density in the lumbar spine by ~2% over a period of 6 months. Furthermore, Far Eastern women are reported to have significantly lower incidence of postmenopausal hot flushes and night sweating compared to Western women, probably because soy food is an important part of their diet (Albertazzi and others 1998). Yet the suggestion that soy food consumption may substitute for hormone replacement therapy (HRT) may require further study. Recent research has shown that not only soybean seeds, but also soybean leaf powder and ethanol extracts of soybean leaf, exert cardio-protective effects by modulating serum lipid profiles (Ho and others 2003). These findings are very interesting, but, again, more research is needed.

40.5.1.4 Phytosterols. FDA expanded the scope of a phytosterol heart-health claim in February 2003. The agency allowed a broader range of food products and dietary supplements to bear the heart health claim in labeling when they are formulated with 0.65 g of phytosterol ester or 0.4 g of free phytosterol per serving (www.cfsan.fda.gov/~dms/ds-ltr30.html).

40.5.1.5 Fiber. FDA adopted a final rule to a regulation authorizing a health claim for the role of β-glucan from whole oat sources in reducing the risk of coronary heart disease (CHD) in July 2003 (FDA, Federal Register of July 28, 2003 [68FR 44207–44209]).

40.5.1.6 D-tagatose. FDA authorized a dental health claim for D-tagatose in July 28, 2003 (FDA, Federal Register of 3 July, 2003 [68 FR 44207–44209]).

40.5.2 Qualified Health Claims

In July 2003 FDA began allowing qualified health claims for food in an effort to make it easier for food manufacturers to make health benefit claims for their products. Even if the scientific evidences in support of the claims are not conclusive, FDA has been helping consumers to obtain accurate, up-to-date science-based information about the health effects of these products. Qualified health claims have been approved for a number of foods, and conditions for their use have been specified (www.cfsan.fda.gov/~dms/hclmgui3.html, www.cfsan.fda.gov/~dms/qhc-sum.html). These foods and their nutraceutical components, and diseases against which they appear to be effective, are itemized in the following sections.

40.5.2.1 Selenium and Cancer. Selenium may help to prevent certain kinds of cancer. However, FDA has determined that the evidence for this is limited and inconclusive. Eligible foods are dietary supplements containing selenium.

40.5.2.2 Antioxidant Vitamins and Cancer. Some scientific evidence suggests that consumption of antioxidant vitamins may reduce the risk of developing certain kinds of cancer. However, the evidence is limited and is not conclusive. Eligible foods are dietary supplements containing vitamin E and/or vitamin C.

40.5.2.3 Nuts and Heart Disease. Scientific evidence suggests, but does not prove, that eating about 46.51 g (1.5 oz.) per day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease. Eligible foods are whole or chopped almonds, hazelnuts, peanuts, pecans, pine nuts, pistachio, Brazil nuts, and walnuts, or foods containing one or more of these nuts.

40.5.2.4 Omega-3 Fatty Acids and Coronary Heart Disease. Consumption of omega-3 fatty acids may reduce the risk of coronary heart disease. FDA evaluated the data and determined that, although there is scientific evidence supporting the claim, the evidence is not conclusive. Eligible foods are dietary supplements containing the omega-3 long-chain polyunsaturated fatty acids EPA and/or DHA.

40.5.2.5 Phosphatidylserine and Cognitive Dysfunction and Dementia. Consumption of phosphatidylserine may reduce the risk of dementia in the elderly. However, FDA concludes that there is little scientific evidence in support of this claim. Eligible foods are dietary supplements containing phosphatidylserine.

40.5.2.6 Folic Acid and Neural Tube Birth Defects. A level of 0.8 mg folic acid in a dietary supplement is more effective in reducing the risk of neural tube defects than lower amounts present naturally in foods. FDA does not endorse this claim yet. Eligible foods are dietary supplements containing folic acid.

40.6 FUNCTIONAL FOODS AND NUTRACEUTICALS: SAFETY ISSUES

As functional foods and nutraceuticals are being marketed in ever-increasing quantities for human consumption, it is absolutely essential to ensure that they are safe, and that they

meet high standards of quality. The biologically active components have to be identified and quantified, and the manufacturers must strive for consistency of product quality and levels of active components in the products. Above all, clinical studies must verify the safety and efficacy of the products, and must establish the proper effective dosage for the treatment of particular diseases and conditions. The optimal dosage has to be defined, as the dosage must be high enough to be effective, yet not so high that it is hazardous or causes adverse side effects or interacts in undesirable ways with other functional foods or nutraceuticals, or with prescription or nonprescription drugs.

It is highly advisable to consult a physician before planning to take any nutraceuticals or herbal supplements, especially if one has certain medical conditions, such as high blood pressure, thyroid dysfunction, psychiatric disorders, Parkinson's disease, blood clotting problems, diabetes, heart disease, epilepsy, glaucoma, or a history of stroke. Furthermore, consumers should not assume that functional foods and nutraceuticals are safe merely because they are labeled "natural". It is important to recognize the fact that

1. Functional foods and nutraceuticals may have harmful side effects in at least some individual cases, and
2. These products may tend to cancel or amplify the function of a prescription drug or over-the-counter drug, causing adverse health effects (American Academy of Family Physicians 2003; Mayo Clinic 2003).

Unfortunately, testing is not, at present, required for herbal health products, as these products are not subject to government regulations pertaining to product safety and standardization of quality and concentrations of active components.

Some examples of the problems that may arise through consumption of functional foods or nutraceuticals along with drugs are given in the following (American Academy of Family Physicians 2003; Mayo Clinic 2003):

40.6.1 Echinacea

Taking echinacea with anabolic steroids or medicines for treatment of irregular heartbeat, rheumatoid arthritis, or fungal infections should be avoided. Echinacea should not be combined with certain other drugs, as it may

1. Cause liver damage;
2. Stimulate the immune system and interfere with effects of immunosuppressants,
3. Raise the levels of HIV protease inhibitors and calcium channel blockers; or
4. Produce undesirable side effects when combined with anti-anxiety drugs.

40.6.2 Ephedra

(Also called "ma huang", "herbal ecstasy", or "mahuanggen.") Taking Ephedra products with caffeine, decongestants, stimulants, heart drugs, antidepressants, or asthma inhalers should be avoided. They can cause high blood pressure or uneven heartbeat, nervousness, or headache, and can lead to potentially fatal heart attacks or strokes.

40.6.3 Feverfew

Feverfew should not be taken with aspirin or other medicines that interfere with the clotting of blood. Feverfew may enhance this effect, causing spontaneous and excessive bleeding.

40.6.4 Garlic

Garlic should not be taken with aspirin or other medicines that interfere with the clotting of blood, or with immunosuppressants or HIV protease inhibitors. Combining garlic and anticoagulant medication may cause spontaneous and excessive bleeding. Besides, garlic may decrease the effectiveness of immunosuppressants and HIV protease inhibitors.

40.6.5 Ginger

Ginger should not be taken with aspirin or other medicines that interfere with the clotting of blood or with H2 blockers or acid-blocking medication. Ginger may increase the effect of anticoagulants and also increase the production of stomach acid, which would counteract the effect of anti-acid medications. On the other hand, ginger may lower the blood pressure or blood sugar levels, which may reduce the need for blood-pressure-lowering medicines or insulin.

40.6.6 *Gingko Biloba*

Gingko biloba should not be taken with aspirin or other medicines that interfere with the clotting of blood or with antidepressant or antipsychotic medicines, or insulin. It may increase the anticoagulant effect of these drugs and can cause bleeding as a side effect. *Gingko biloba* can also increase the levels of antidepressant drugs in the blood. When combined with antipsychotic drugs, it may cause seizures. In addition, it may affect insulin levels.

40.6.7 Ginseng

Ginseng should not be taken with aspirin or other medicines that interfere with the clotting of blood or with antidepressants, heart medication, insulin, or oral antidiabetic medicines. Ginseng combined with warfarin (coumadin) can increase the risk of excessive spontaneous bleeding. It can also interfere with digoxin's pharmacologic action. Moreover, it can reduce blood sugar levels in people with type-2 diabetes (requiring especially careful glucose-level monitoring).

40.6.8 Kava Kava Products

Kava kava products should not be taken with sedatives, sleeping pills, antipsychotic drugs, alcohol, or drugs for anxiety or Parkinson's disease. When combined with any of these drugs, kava kava can induce deep sedation and, in some cases, even coma. Liver damage resulting from the use of kava kava was reported in Europe in late 2001. FDA subsequently undertook an investigation of the product's safety.

40.6.9 St. John's Wort

St. John's wort should not be taken with alcohol or with any prescription medicines such as antidepressants, digoxin, asthma medication, oral contraceptives, blood-thinning medication, Tamoxifen, drugs for controlling blood pressure, or medicines for heart disease. St. John's wort affects the body's metabolism of these substances.

In conclusion, the benefits and risks of consuming physiologically active functional foods and nutraceuticals have to be balanced carefully, because

1. Biological responses may differ widely among different individuals;
2. Adverse effects are possible in some cases, and

3. These substances may interfere with the effects of prescription or nonprescription drugs or other functional foods and nutraceuticals.

Systematic regulation and quality control of functional foods and nutraceuticals marketed for human consumption are essential to public health and well-being.

40.7 REGULATION OF FUNCTIONAL FOODS AND NUTRACEUTICALS

There has been growing recognition of the key role of diet in disease prevention and treatment. Thus, the production and consumption of functional foods and nutraceuticals, the popular demand for them, and realization of their importance in promoting good health, have increased greatly in recent years. Accordingly, for manufacturers and consumers alike, there must be well-defined, science-based standards and regulations to ensure that these products have consistently high quality, that their components are identified and accurately quantified, and that they are safe (Stephen 1998). Product labels must list not only the components and their concentrations, but also warnings about possible adverse effects and interactions or interference with other substances, such as drugs and food supplements. In addition, stringent clinical trials must be performed to investigate the beneficial effects, efficacy, and adverse effects of the products, to establish the proper dosage for the treatment or prevention of specific medical conditions, to determine the range of doses that are safe, and to study the consequences of combining them with drugs and other substances.

Regulatory policies for functional foods and nutraceuticals are not well established in most countries of the world, except for Japan (see later), as there are so many different types of products with different health benefits. Moreover, there is considerable confusion about "what these products should be called, how they should be regulated, whether or not they raise safety concerns, and whether or not health claims should be permitted and so on" (Stephen 1998). In brief, the different national policies regarding functional foods and nutraceuticals in Canada, the European Union, Japan, the United States, Australia and New Zealand, are described in the following.

In its Food and Drug Act, Canada has, thus far, recognized no separate category for functional foods or nutraceuticals. Some "biological role claims", however, have been approved for certain foods through the Food Directorate. To gain official recognition of an effect of such a product on a specific disorder or disease (whether the product is eaten as food or prepared as a concentrate), an application for approval as a drug must be submitted under the Therapeutic Products Programme of the Health Protection Branch of Health Canada (Scott and others 1996; Smith and others 1996; Stephen 1998). However, advertising the supposed effects of functional foods and nutraceuticals on selected diseases and disorders specified in a list called "Schedule A" (e.g., hypertension, diabetes, arteriosclerosis, cancer, arthritis, and obesity) is prohibited. For instance, the manufacturer of a functional food or nutraceutical that tends to lower cholesterol levels is not allowed to publicize this effect (Smith and others 1996). The current system in Canada offers manufacturers the option of licensing and selling such products as "drugs" in accordance with the definition of drugs in the Food and Drug Act. This process is very expensive and imposes severe restrictions on marketing. Apart from that, most functional foods and nutraceuticals in Canada are marketed without regulation. Nevertheless, this situation has changed as Health Canada issued a revised Nature Health Product Policy in July 2003 and its regulation came into effect as of 1 January, 2004 (updated 20 October, 2006).

In contrast, the regulatory system of Japan has been supportive of the development and marketing of functional foods. The Ministries of Agriculture and Health and Welfare joined forces to develop a comprehensive policy to promote the manufacture of food components that confer potential health benefits. This policy is rooted in traditional Oriental lore, which holds that many foods and food constituents are effective in the prevention and treatment of disease. Accordingly, in 1991 the government established a system called "Foods for Specified Health Use" (FOSHU) for the purpose of licensing such substances (Stephen 1998). Their objective, above all, was to maximize the health of the growing numbers of elderly people in the population (Lapsley 1996). It has evolved through close collaboration among food industries, government, academics, and research organizations. Thus, accredited food industry organizations work closely with government agencies and food companies, and play a direct role in the regulation of functional food products under the regulations of FOSHU. The licensing process is clearly defined and is managed jointly by government and industry. As a result, a functional food is evaluated, and food manufacturers may make health claims for it. On being licensed and receiving an official FOSHU seal of approval, these foods may then be sold on the domestic market, and consumers have confidence in them. Currently there are over 150 FOSHU-approved products available in Japan.

In the 15 nations of the European Union, there are a number of initiatives concerning functional foods, but they appear to concentrate mainly on relevant scientific issues (Smith and others 1996; Stephen 1998). Currently, regulation of functional foods, food supplements, and/or nutraceuticals is voluntary and is therefore left to the discretion of each member country. There are no universal standards, regulations, or policies pertaining to such products. Therefore, the systems of regulation vary widely from one member nation to another, and many countries consider these products simply as foods. Claims relating to a disease are generally not permitted. If such claims are made, however, the products come under the regulations for drugs and other medicinal substances.

In the United States, there is no separate category or set of regulations for functional foods and nutraceuticals. These products fall under the regulations for conventional foods. They must be safe to be marketed as foods, and the ingredients must be "generally recognized as safe" (GRAS) or approved as food additives. Nevertheless, there is a distinction between dietary supplements and foods. For a new ingredient to be approved for use as a dietary supplement, it is submitted to the FDA 75 days before the supplement is to be put on the market. FDA then evaluates it and either accepts or rejects it on the basis of safety data provided by the producer. Where foods are concerned, however, if an ingredient is not already GRAS, it takes a long time to be declared GRAS. There are no definite rules to guide this process, and no clear standards for judging the data provided by companies. Having functional foods approved as GRAS is not a simple task.

Regarding botanicals used as food ingredients, the fundamental issues are the identity of the plant and identification and quantification of the phytochemicals in the plants. To settle the first issue, harvesting the correct genus of the plant is essential.

In Australia, the Parliamentary Secretary to the Minister for Health and Ageing Consumers recently released a policy statement called Food Standards Australia–New Zealand (FSANZ) – Food Labeling Issues, after a survey of consumers in Australia and New Zealand. The key findings of the survey were that the most commonly examined specifications on the labels were the "by" or "best before" dates, lists of ingredients, and information about health claims, allergens, GMOs, and novel or irradiated food declarations. The research also revealed that consumers want further information to help them to understand the basics of healthy eating and to make healthy choices of foods by

following the guidelines provided by food labels. As a result, FSANZ has undertaken to work with food industry, health professionals and educators to ensure that this information is available. This survey was very timely, as it was conducted just before December 2002, when the New Food Standards Code came into full force (www.foodstandards.gov.au).

40.8 PUBLIC EDUCATION AND DIETARY GUIDANCE

As a party to the Consumer Health Information for Better Nutrition Initiative of the U.S. Government, FDA is seeking ways to promote inclusion of dietary guidance statements on food labels. The agency is encouraging good nutrition among consumers in a variety of ways, including food manufacturers' dietary guidance statements, which are available from Federal government agencies. The National Cancer Institute (NCI), in a collaborative effort with FDA, recently issued an important dietary guidance message for consumers: "Diets rich in fruits and vegetables may reduce the risk of some types of cancer and other chronic diseases." Food manufacturers may also use this statement on food labels along with the new "5-to-9-Day" logo. FDA has pointed out that this statement about fruits and vegetables is only a "dietary guidance statement" and not a "health claim", emphasizing, however, that dietary guidance statements must be truthful and not misleading. FDA works cooperatively with Federal agencies on an ongoing basis to encourage the development and use of dietary guidance messages (FDA 2003). An example is FDA's small entity compliance guide (SECG) for a final rule on food labeling published in the Federal Register of July 11, 2003, entitled "Trans Fatty acids in Nutrient Content Claims, and Health Claims". Alternatively, this SECG is called "Food Labeling – Trans Fatty Acids in Nutrition Labeling, Nutrient Content Claims and Health Claims" (FDA, Federal Register, 68 (161), 50155–50156).

The average American eats over 4 g of salt a day. In order to reduce possible harmful effects of hidden salt in food, the National Institute of Health (NIH) advises healthy people to have no more than 2.4 g of salt (as sodium chloride) a day and 1.5 g for those with high blood pressure. Salt is concealed in common foods, especially restaurant foods and processed foods. Too much sodium increases the blood pressure, and high blood pressure has adverse effects on the heart, brain, and kidneys. The American Public Health Association, backed by many other health-care and medical groups, urge the processed food industries and restaurants to lower the amounts of salt in their foods (NIH 2003).

Another example of hidden food components that may be harmful is gluten, a protein found in wheat and related cereal grains. About 10–15% of the American population cannot tolerate gluten; indeed, gluten is actually toxic to some individuals. Today's consumers must check food labels to detect hidden gluten. They must be more cautious than ever nowadays, as gluten is found in more than 40,000 supermarket products, compared with 7000 products in the 1960s (www.glutenfree101.com).

A survey carried out jointly by the Food Marketing Institute (FMI) and Prevention magazine revealed that the choice of food purchases made by families with children depends mainly on convenience. However, most consumers would like to have more information to help them to eat more healthful foods and have a balanced diet (http://www.ift.org/ and http://www.fmi.org/mediatext.cfm?id = 565). In the United States, nearly 65% of adults and 15% of young people aged 6 to 19 are overweight, and 31% of the adults are classified as obese according to the National Center for Health Statistics of the Centers for Disease Control and Prevention. An even more alarming fact is that the estimated number

of overweight children and adolescents has nearly tripled in the past two decades according to the U.S. Surgeon General. The new survey showed that 74% of households with children and 63% with no children admit that their diets could be healthier. The survey results revealed the following primary reasons that the subjects did not have healthier diets:

1. They were "too busy" to eat healthy foods, they had no time to cook at home, and a third of the women worked outside the home.
2. "Healthy fast foods are hard to find." One third of shoppers would like to see a greater variety of healthy foods on the menus of fast-food restaurants.
3. "Healthy foods cost too much."
4. Friends and family members "do not care" about healthy eating habits, educating friends and family members about nutrition and the necessity of eating balanced meals to maintain good health is important.
5. There is "confusion about health claims"; consumers are confused about what qualifies as a healthy food product and what they should eat to have healthy and balanced diet (http://www.fmi.org/mediatext.cfm?id=565).

40.9 CONCLUSIONS

In recent years there has been enormous progress in the study of functional foods and nutraceuticals and their importance to the health of humans and animals, but there is a need for further research in many areas of this ancient, yet modern, field. To begin with, there is ample scope for more basic research that will lead to a better understanding of the mechanisms by which functional foods and nutraceuticals exert their beneficial (and, in certain cases, harmful) effects. This, in turn, should lead to advances in the applied aspects of the field. Thus the biologically active components must be identified and quantified using rigorous, standardized, internationally accepted methods. Concomitantly, clinical and epidemiological investigations are required to assess the effects of functional foods and nutraceuticals on human health, with attention not only to their efficacy but also to issues such as proper dosage, delivery methods, bioavailability, and safety (for instance, possible adverse effects arising from allergies or interference with prescription or over-the-counter pharmaceuticals or with other functional foods and nutraceuticals). Another important research area is exploration for the purpose of discovering as yet unknown medicinally beneficial substances in plants and other organisms (e.g., marine algae and animals). It is essential, however, that the discovery and subsequent exploitation of new sources of functional foods and nutraceuticals in nature be accomplished without damaging the environment or depleting populations of wild species.

Furthermore, the field of functional foods and nutraceuticals has political, legal, and administrative dimensions. Thus, laws and government policies, regulations, and guidelines must be established to authorize the marketing, labeling, and advertising of specific products on the basis of scientifically sound criteria for evaluating their efficacy, safety, and quality. The field is economically important as well. The production, processing, and marketing of functional foods are a thriving, multifaceted business. The sale of such products has expanded greatly in the recent past to meet rising public demand for such products as a consequence of increasing awareness of their beneficial effects on human health. A less direct but equally significant economic issue is the reduction in the cost of health-care that may be expected if public health is benefited by improvements in eating habits.

Finally, public education about functional foods and nutraceuticals is another vitally important aspect of this field. A healthful diet helps to prevent and ameliorate the chronic diseases that are so prevalent in modern society. Yet, poor eating habits and their inevitable harmful consequences constitute a serious, widespread problem. The more thoughtful members of the general public have become increasingly concerned about dietary issues, increasingly aware of the crucial importance of a balanced, healthful diet, and increasingly knowledgeable about the good health and improved quality of life that may result from regular consumption of functional foods and nutraceuticals.

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41

Functional Foods: International Considerations

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41.1 Introduction 940
41.1.1 Functional Foods 940
41.1.1.1 United States 941
41.1.1.2 Japan 941
41.1.1.3 Europe 941
41.1.2 Dietary Supplements 941
41.1.2.1 United States 941
41.1.2.2 Canada 941
41.1.2.3 Europe 942
41.1.2.4 Japan 942
41.2 The Global Market 942
41.3 Macro Trends Affecting the Functional Food Industry 943
41.3.1 The Rising Cost of Health-Care Related to Chronic Disease 943
41.3.2 The Role of Food in Prevention Strategies 945
41.3.3 Safety and Toxicology Aspects Related to Functional Foods and Nutraceuticals 945
41.3.4 Globalization of the Food Industry 946
41.3.5 Consumers' Focus on Prevention of Illness 947
41.4 United States 948
41.4.1 Market 948
41.4.2 Regulatory Review 948
41.5 European Union 950
41.5.1 Market 950
41.5.2 Regulatory Review 950

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

41.6 Japan 952
41.6.1 Market 952
41.6.2 Regulatory Review 952
41.7 The Global Business of Functional Foods and Nutraceuticals 953
41.8 Conclusions 953
References 954

41.1 INTRODUCTION

In the late 1980s, consumers began to recognize that certain foods and food supplements could have an impact on health. The first phase of this "health and wellness revolution" belongs to dietary supplements, where, through the passage of the Dietary Supplement Health and Education Act (DSHEA) in the United States in 1994, market dynamics changed significantly – initially to the positive and then to the negative (United States Food and Drug Administration 2004).

For the food industry, it has been a somewhat different story, which has yet to play itself out fully. The United States responded to industry pressure in 1990 by enacting the Nutrition Labeling and Education Act (NLEA) to allow manufacturers to promote the benefits of their food products (United States Food and Drug Administration 2003). This regulatory framework catalyzed the functional foods revolution in the North American marketplace, which welcomed new food product introductions, and a revival of science and consumer interest.

The global market for functional foods and ingredients is comprised of companies that see the industry as a growth opportunity and the ability to make higher margins. It has attracted players the size of BASF, DSM, Nestle, Cargill, Bayer, and Danone, as well many small but growing small- and medium-sized enterprises.

41.1.1 Functional Foods

In many respects, comparing the industry across regions is challenging due to the various definitions, and thus scope, of functional foods, dietary supplements and natural health products throughout the world. "Functional foods" is essentially a marketers' or analysts' term and globally is not recognized in law or defined in the dictionary. For the purposes of this chapter, the definition of functional foods as proposed by Health Canada will be used. In 1998, it was proposed that a functional food was "... similar in appearance to a conventional food, consumed as part of the usual diet, with demonstrated physiological benefits, and/or to reduce the risk of chronic disease beyond basic nutritional functions" (Health Canada 2000).

As a category, functional foods include:

- Conventional foods containing naturally occurring bioactive substances (such as dietary fiber in wheat bran to promote digestive regularity, or β-glucan in oat bran to lower blood cholesterol);
- Foods that have been modified, by enrichment or other means, in terms of the amount, type, or nature of their bioactive substances. An example is margarine that contains added phytosterol, an extract from plant sources that is known to interfere with cholesterol absorption, thereby lowering serum cholesterol levels; and
- Synthesized food ingredients, such as some specialized carbohydrates intended to feed microorganisms in the gut.

41.1.1.1 United States. The most commonly referenced definition for a functional food in the United States is that used by California-based Nutrition Business Journal (NBJ) (Anon 2003). NBJ defines a functional food as fortified with added or concentrated ingredients and/or marketed to emphasize "functionality" to improve health or performance. Unlike Health Canada's definition, NBJ includes "substantially fortified", "inherently functional", and "performance" foods within their definition.

41.1.1.2 Japan. Japan is the only country with a regulatory framework for functional foods. Foods for Specified Health Uses (FOSHU) are defined as those to which a functional ingredient has been added for a specific health effect, designed to promote or maintain good health. Under FOSHU such foods include those that contain functional substances that affect the physiological function and biological activities of the body; those that claim if used in the daily diet, one can hope for a specified health benefit. Under the legislation, such foods must be evaluated individually and approved by the government. FOSHU can also be used for dietary supplements (Japan Health Food and Nutrition Food Association 2004).

41.1.1.3 Europe. As in North America, several definitions of functional foods are used throughout the 15 countries of the European Union. The most widely accepted is "Foods that by virtue of physiologically active food components provide health benefits beyond basic nutrition" (Fielding and Frayn 1998).

41.1.2 Dietary Supplements

41.1.2.1 United States. In 1994, the Dietary Supplement Health and Education Act (DSHEA) was passed in the United States as an amendment to the Federal Food Drug and Cosmetic Act (United States Food and Drug Administration 2004). DSHEA regulates dietary supplements, which are defined as "a product, other than tobacco, intended to supplement the diet that contains at least one or more of the following ingredients: a vitamin, a mineral, a herb or other botanical, an amino acid, or a dietary substance for use to supplement the diet by increasing the total dietary intake; or a concentrate, metabolite, constituent, or extract or combination of any of the previously mentioned ingredients".

41.1.2.2 Canada. Canada is the only global jurisdiction that has legislation related to "natural health products (NHP)". These regulations became effective on January 1, 2004 (Government of Canada 2003). The new regulations apply to all NHPs, including homeopathic preparations, substances used in traditional medicine, a mineral or trace element, a vitamin, an amino acid, an essential fatty acid or other botanical, animal or microorganism-derived substance. These products are generally sold in a medicinal or "dosage" form. Until publication of these regulations, the working definition for a nutraceutical in Canada has been "a product that has been isolated or purified from foods and generally sold in medicinal forms not usually associated with food. Nutraceuticals have been shown to exhibit a physiological benefit or provide protection against chronic disease". Health Canada decided that the product category of nutraceuticals would be encompassed within NHP regulations.

However, like functional foods, although the nutraceutical category is not recognized in law, it is used extensively as a marketing term for plant and animal based bioactives and ingredients that are sold in a medicinal form.

41.1.2.3 Europe. In the EU, dietary supplements are referred to as "food supplements", the purpose of which are to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form (European Parliament 2002). Such dosages are capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities.

The EU adopted Directive 2002/46/EC on June 10, 2002, with the requirement that it be passed into national laws by all member states by July 31, 2003 (Hanssen 2004). It deals with laws relating to food supplements. The nutrients that are included are restricted to vitamins and minerals. Additional nutrients such as amino acids, essential fatty acids and fiber may be added later. For countries where products currently exist that included ingredients not yet on the EU Directive List, these will be able to continue to be marketed, but not permitted for EU-wide use. Until January 31, 2009, the authorities of the European Union Member States may provide derogations for vitamins and minerals and their forms not included in the Food Supplements Directive, as long as the following conditions are fulfilled:

- The substance in question was used in food supplements marketed in the Community prior to July 12, 2002;
- The European Food Safety Authority has not given an unfavorable opinion in respect of the use of the substance (European Commission 2006).

41.1.2.4 Japan. Since 2001, 13 vitamins, 13 minerals, and 101 herbal supplements have been regulated within the food category, rather than as drugs. In April 2001, Japan implemented new regulations allowing dietary supplement labels to provide health and efficacy information for the first time. Dietary supplements may now carry claims that are regulated under FOSHU or Nutrient content claims. Herbals that carry health claims are considered within the more stringent FOSHU regulations. Currently, 12 vitamins and 2 minerals have been placed with the nutrient content category. This area represents 20% of the nutritional supplements market, and the remaining 80% is classified as either FOSHU (requiring individual approvals for health claims) or "other food" for which no health or efficacy claims are allowed.

With regard to market segregation, functional foods and dietary supplements lie between the health-care continuum of foods and drugs as indicated in Figure 41.1 (Anon 2003).

41.2 THE GLOBAL MARKET

For 2004, the Nutrition Business Journal (NBJ) estimated current world consumption of dietary supplements and functional foods to be approximately US $182 billion. The

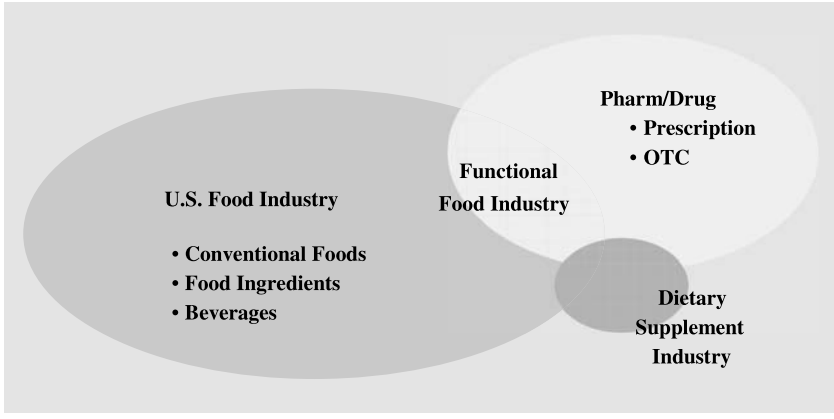

Figure 41.1 *Context for functional foods and dietary supplements.*

primary markets for supplements and functional foods are the United States, Europe, Japan, and Asia (Ferrier 2004). These regions are, for the most part, characterized by greater levels of economic development and more sophisticated economies. In the case of Asia, historical consumption patterns play a role.

The 2004 global functional food market is valued at over $70 billion. The United States, Europe, and Japan are the major markets for functional foods, capturing approximately 30% each, similar to the overall market capture for total sales in all nutrition categories. The United States is the most dynamic market for functional foods, with a growth rate of more than triple that of conventional foods at $\sim$9–12% per annum. It is anticipated that, based upon past data, the industry will witness steady and continuous growth to 2008, when projections of sales for functional foods globally near $95 billion (Ferrier 2005).

41.3 MACRO TRENDS AFFECTING THE FUNCTIONAL FOOD INDUSTRY

Several macro trends are driving the growth of the global functional foods industry.

41.3.1 The Rising Cost of Health-Care Related to Chronic Disease

The World Health Organization's 2003 report on diet, nutrition and the prevention of chronic disease noted that, "the burden of chronic disease is rapidly increasing worldwide" (WHO 2003). In 2001, chronic diseases contributed approximately 60% of all deaths worldwide and 46% of the total burden of disease. Almost half of these deaths are from cardiovascular diseases, obesity and diabetes. Increasingly, these diseases are also becoming more prevalent in developing countries. The WHO estimates that chronic disease will account for 70% of all deaths worldwide by 2020.

An increase in disease incidence leads to overall increases in the cost of health-care. In most developed countries these costs average between 9 and 14% of the gross national product (GNP). The United States, in particular, is facing steep increases in the cost of

delivering health-care. Along with the United States, Canada is among the top three of the G7 countries in terms of the cost of health-care per capita.

In the United States, health-care costs were $1.6 trillion in 2003 and are projected to reach $3.1 trillion by 2012 – or 17.7% of GNP. Spending by private health insurance has increased 10-fold since 1987. Costs increased from $3.6 billion in 1987 to $36.5 billion on obesity-linked illnesses in 2002. Total health-care spending on obesity in 1987 was 2% compared to 11.6% in 2002. The rise in treated disease prevalence, rather than the rise in spending per treated case, was the most important determinant of spending growth (Thorpe and others 2005).

According to Health Canada, in 2003 Canada spent approximately $121 billion on health-care (averaging $3839 per person) (Public Health Agency of Canada, Economic Burden of Illness in Canada, 2004). It is estimated that Canada's health-care system will cost $1.4 trillion by 2015 (Lussier 2005). Chronic disease accounts for a significant portion of that burden. Cardiovascular diseases, diabetes, and cancer, combined, cost the Canadian economy more than $55 billion annually (Public Health Agency of Canada 2002). As a specific example, Canada's health-care burden is expected to increase as a result of obesity in Canada. The direct cost of obesity to Canada's health-care system is estimated to be $1.8 billion (Heart and Stroke Canada 2006).

In part, the increase in health-care costs is related to an aging population. During the twentieth century, the number of Americans aged 65 years and above increased 1100%. By the year 2035, it is estimated that 70 million people will be in this age bracket (U.S. Census Bureau 2005). By 2010, the number of people over 50 years old will increase by 48%; in contrast, the group aged 13–24 years will increase by only 16%.

Currently, more than 75% of those over 65 years old suffer at least one chronic disease, whereas 50% have at least two, a situation that imposes a tremendous burden on the health-care system.

Dietary intervention is one pillar within a successful health-care mandate. Any effective dietary intervention policy must begin in early life and stress the importance of a life-style approach. Governments are attempting to develop measurements to benchmark the dietary practices of the population to identify areas for improvements and to track changes in disease incidence as a result of health policy initiatives. The Japanese government has been very successful in implementing polices that capture these elements.

The World Health Organization has created new guidelines for governments in an attempt to reduce global chronic diseases (WHO 2005). The guidelines are featured in a comprehensive survey, "Preventing Chronic Diseases: a vital investment", which charts the rise of preventable deaths from heart disease, stroke, cancer, and diabetes in nine EU countries. In the report, 80%, or 36 million, deaths from heart disease, stroke, cancer, and adult-onset diabetes could be prevented through life-style modifications, over the next 10 years.

The report calls for a series of measures to be introduced to reach the target of cutting deaths by 2% per year by 2015. These include limiting the availability of high-fat, high-salt, and high-sugar foods, while other milestones are intended to encourage governments to implement food-labeling legislation and increase regulation of food marketing aimed at children.

Although the strategy is not enforceable, the surrounding debate generated by this and other reports on rising obesity in the world continues to put pressure on the food industry to offer healthier foods.

41.3.2 The Role of Food in Prevention Strategies

There is limited information regarding the impact of nutritional strategies in disease prevention and health-care cost reduction. Some case studies, however, are available that support the use of functional foods to improve the health of populations.

According to Unilever, a reduction of LDL cholesterol by 14% through the consumption of a cholesterol-lowering margarine, if sustained over a five-year period, could result in a decrease in coronary risk in the UK population of around 25% (Unilever UK 2001). If this risk reduction were achieved in practice, this would reduce UK heart disease patient numbers by 250,000 and save the UK health-care system £433 million.

One of the principal examples is the introduction of margarine spreads fortified with plant sterols in the United Kingdom. The UK National Health Service estimated that these products have the potential to lower health-care costs for cardiovascular disease by £100 million per year. An added benefit to this cost savings is that fact that this reduction can be delivered at an annual cost of only £70 per patient per annum, which is borne at the expense of the patient and not the government (UK National Health Service 2000).

In Canada, Holub (2002) suggests that there is a potential for the Canadian government to save an approximate $19 billion in health-care costs per annum with the introduction of several functional foods and nutraceutical ingredients, including citrus pectins, guar gum, plant sterols, long-chain omega-3 polyunsaturated fatty acids, cholestin (red rice yeast) and policosanol into the diet.

41.3.3 Safety and Toxicology Aspects Related to Functional Foods and Nutraceuticals

Functional ingredients, although intended to produce a physiological effect to reduce chronic disease risk or otherwise optimize health, may also produce adverse effects under certain circumstances due to the fact that they encompass elements of drugs, nutrients, and food additives (Kruger and Mann 2003). Thus, assurance of safety for such ingredients is critical, particularly because the general population consumes them in an unsupervised manner (i.e., without medical oversight). When evaluating the safety of functional ingredients, clinical substantiation of safety is critical and, in particular, whether current or historical human exposure is associated with an adverse health outcome.

In the United States, health ingredients can be marketed as dietary supplements, food additives, or as generally recognized as safe (GRAS) ingredients. The regulatory requirements to determine safety are different for each of these categories. In 1997, the Food and Drug Administration (FDA) proposed replacing the current GRAS affirmation process with a notification procedure by which any person may notify FDA of a determination that a particular use of a substance is GRAS. Under the proposed notification procedure, the FDA evaluates whether the submitted notice provides a sufficient basis for a GRAS determination and whether information in the notice or otherwise available to FDA raises issues that lead the agency to question whether use of the substance is GRAS. Notification is not mandatory if the sponsor chooses to inform the FDA of its GRAS determination. The FDA does not make its own determination of the GRAS status of an ingredient. A substance is GRAS if its use in food has a proven track record of safety based either on a history of use before 1958 or on published scientific evidence (Kruger and Mann 2003).

Under DSHEA, no requirement for pre-market approval is required for dietary supplements, which include "vitamins, minerals, herbs or other botanicals, amino acids, or

other dietary substance[s] for use by man to supplement the diet . . . including concentrate, metabolites, constituents, extracts, or any combination . . . ". Such ingredients must not present "a significant or unreasonable risk of illness or injury" a standard that has been criticized by some experts as reducing the criteria of safety compared with food additives or GRAS ingredients (Burdock 2000).

There are several issues critical to the evaluation of the safety of a functional ingredient (Kruger and Mann 2003). First, due to the fact that these ingredients are physiologically active, mechanisms of action for pharmacologic activity are essential to determine consequences of exposure at varying doses. Secondly, functional ingredients can be single compounds, complex mixtures, or products derived from novel sources/processes – each of which presents unique safety issues, which must be assessed on a case-by-case basis. Thirdly, potential exposure from a functional ingredient must be compared to its determined safe level of intake. Finally, potential adverse drug–food interactions must be assessed.

Although a functional ingredient is intended to produce optimized consumer health, because there is potential for lifetime exposure and consumption is unsupervised, the assurance of safety is critical. The primary measure of safety must be derived from well-controlled randomized, double-blind human clinical intervention trials. Safety must encompass an understanding of the physiologic activity of the functional component, not only as it relates to a potential health benefit, but any potential toxicological effect that may result.

41.3.4 Globalization of the Food Industry

For global survival, more companies are experiencing corporate mergers and acquisitions. As companies seek to gain market share, the functional food market has become increasingly attractive, with larger margins than the conventional food industry and in some regions triple the growth rate. Many large multinational companies see the functional food industry as a way to escape their product being considered a commodity, as the profit margins in functional foods can be between 5 and 6% and sometimes higher, whereas in conventional food products the margins can be as low as 1–2%.

The globalization trend is very evident in Europe, where currently 30 of the largest food companies control 70% of the market compared with 51% of the market in 1992. M + M Planet Retail market research predicts that Europe's top 30 companies could dominate 80–90% of the grocery trade in a few years. This is in part due to the fact that Europe is a contained market where companies from global regions have difficulty in competing as successfully as European companies (AROQ 2003).

The European model for the consolidation of food companies is not unique. Table 41.1 provides a list of the top ten food companies, the location of their headquarters, 2002 sales, growth compared to 2001 sales and the type of food products marketed (Prepared Foods 2002).

Like the traditional food industry, the functional food industry is also consolidating. Figure 41.2 depicts the market consolidation that is present in the North American functional food industry, with five companies controlling nearly 40% of the market. The top 50 control nearly 80% of the market (Anon 2003).

At first glance, it would appear that the companies listed in the top ten of North American functional food companies (Table 41.2) are conventional food companies. However, the many factors in play in the global food market are forcing such companies

TABLE 41.1 Global Top Ten Food Companies.

Company	Headquarters	Food Sales Billions $	% Change vs Prior	Food Type
Nestle	Switzerland	$46.2	+20	Diversified
Kraft Foods	Northfield, IL	$38.1	+12	Diversified
ConAgra	Omaha, NB	$27.6	+2	Diversified
PepsiCo	Purchase, NY	$29.9	+32	Beverages, snacks
Unilever	London/Holland	$26.7	+2.5	Diversified
ADM	Decatur, IL	$23.5	+17	Ingredients, grain
Cargill	Minn., MN	$21.5	+3	Grain based
Coca-Cola	Atlanta, GA	$20.0	−2	Beverages
Diageo	London, UK	$16.6	+0.7	Alcoholic beverages
Mars Inc	McLean, VA	$15.3	+7	Confections

to shift their focus to health and wellness in an attempt to remain ahead of the functional food wave and maintain market share (Anon 2003).

Pepsi has announced plans to have half their product portfolio be "more nutritious", while Nestlé is employing a nutrition-focused acquisition strategy to help shift their image to a "wellness and well-being" company rather than food and beverage company. These companies are seeking to improve product portfolios by releasing new products or redeveloping products so that they are perceived as "healthy" products.

41.3.5 Consumers' Focus on Prevention of Illness

Consumers are a critical driving force behind the development of functional food and nutraceuticals. As they strive to maintain good health into old age, they are attempting to take greater control over their health-care needs. With the rising incidence of obesity and the significant increases in the rates of chronic disease, consumers are becoming increasingly aware of the link between diet and medical disorders. Given the serious statistics related to the increasing death rates and of course, the costs of disease, to Western societies, it is no surprise that consumers are seeking alternatives to conventional medicine, which has been dominated for decades by an attitude of "treat" rather than "prevent" illness.

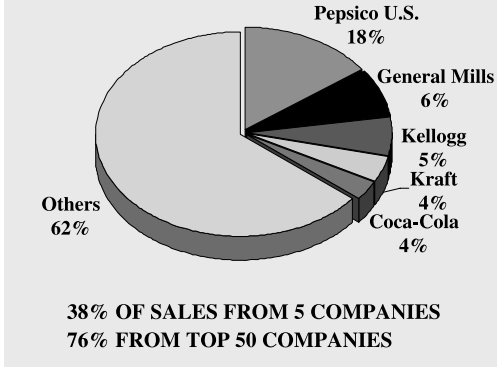

Figure 41.2 *Top functional food companies.*

TABLE 41.2 Top Ten U.S. Functional Food Companies (Sales in $U.S. Billions).

Companies	2000	2001	2002	2001 Growth	2002 Growth
Pepsico U.S.	3113	3441	3806	11%	11%
General Mills	1305	1335	1313	2%	−2%
Kellogg	990	1026	1058	4%	3%
Coca-Cola	565	759	850	42%	12%
Kraft	679	702	738	3%	5%
Red Bull	200	436	690	118%	58%
Campbell Soup	520	559	600	7%	7%
Ocean Spray	455	47	487	5%	2%
Nestle	347	393	459	13%	17%
ConAgra	405	396	415	−2%	5%

41.4 UNITED STATES

41.4.1 Market

In 2004, the sale of nutrition products consisting of dietary supplements and herbs, natural and organic foods, functional foods and natural personal care products, generated $68.6 billion in consumer sales. The dietary supplement market generated $20.9 billion in the United States, growing only 1–3% from 2003. The functional food market grew 9% from sales of $22.7 billion in 2003 to sales of $24.8 billion in 2004, growth generated principally by an expanding range of fortified foods, improved ingredients, and better taste profiles. In comparison, a growth rate of only 1.6% was witnessed for total food sales. Functional foods, however, represent less than 4% of total food sales in the United States. By 2010, however, NBJ expects functional food sales to account for 5.2% of total food sales in the United States, or approximately $31.2 billion (Ferrier 2005). The average rate of growth of the functional foods and dietary supplement segments in the United States is estimated by NBJ to be 6.8% and 4.2%, respectively, for the years 2004 to 2008 (Ferrier 2004).

A key characteristic of the U.S. functional food market is a large focus on disease and its prevention. Products aimed at lowering blood cholesterol levels and cancer risk as well as weight loss, have characterized the U.S. market. The use of botanicals in functional foods is much more popular than in Europe or Japan. Breads and grains, especially enriched breakfast cereals, followed by functional beverages (teas and energy drinks) are the primary functional food products in the U.S. market. Snack foods, including nutrition bars, are one of the fastest growing functional categories, experiencing a 23% rate of growth in 2003 (Ferrier 2004).

41.4.2 Regulatory Review

The ability to link a food or food component to health is based on sound scientific evidence, with the desired standard being replicated, randomized, placebo-controlled, intervention trials in human subjects (Hasler 2002). Once a sound scientific basis has been established for a food, the process for obtaining a health claim for the product can be initiated.

There are three basic types of health claims: structure/function (e.g., calcium helps to build strong bones), risk reduction (e.g., calcium helps reduce the risk and progression of osteoporosis), and therapeutic (e.g., product X is indicated for the treatment of osteoporosis).

Currently the ability to utilize health claims to promote the nutritional value-added attributes associated with functional foods varies internationally.

The United States has a wide variety of fortification policies and health claims labeling, which permit consumers to make informed dietary choices. Health claims that can be used on food and dietary supplement labels fall into three categories: risk reduction claims, nutrient content claims, and structure/function claims. The responsibility for ensuring the validity of these claims rests with the manufacturer, Food and Drug Administration (FDA), or, in the case of advertising, with the Federal Trade Commission (FTC).

The Nutrition Labeling and Education Act (NLEA) of 1990 directed the FDA to change the way that food labels were regulated, in order to make additional nutritional information available to consumers. As defined in this act, a health claim is a "statement that characterizes the relationship of a substance to a disease or a health-related condition, typically in the context that the regular dietary consumption of a substance 'may reduce the risk' of a specific disease or health condition". Within the labeling claims, references that the food is intended to "cure", "mitigate", or "prevent" any disease are not permitted.

In 1994, FDA reviewed several diet–disease relationships and established seven allowable risk reduction health claims that can be made on conventional foods as long as they meet specific nutritional criteria related to fat, saturated fat, cholesterol, sodium, vitamin A, vitamin C, iron, calcium, protein, and fiber.

In January 1997, the FDA approved the first food-specific health claim under the NLEA, in response to a petition from the Quaker Oats Company. The authorized health claim describes the relationship between consumption of whole oat products and coronary heart disease risk reduction.

The FDA Modernization and Accountability Act (FDAMA) became law in November 1997 (U.S. Food and Drug Administration 2001). It contains provisions to reduce the regulatory hurdles in the health claim approval process. Specifically, it directs the FDA to authorize health claims that are based on the published authoritative statements from U.S. Government agencies such as the Centers for Disease Control (CDC), the National Academy of Sciences (NAS), or the National Institutes of Health (NIH). An authoritative statement is "about the relationship between a nutrient and a disease or health-related condition". Within FDAMA, health claims can be made without going through the lengthy FDA review process, if they have already been published by these agencies. Premarket notification to the FDA of 120 days is required. In July 1999, General Mills received no objection for a claim linking whole grain foods to reduced risk of heart disease and cancer, based on statements from the National Academy of Sciences.

In December 2003, the FDA's *Consumer Health Information for Better Nutrition Initiative* was announced (U.S. Food and Drug Administration 2003). The new system allows the use of qualified health claims when there is emerging evidence for a relationship between a food, food component, or dietary supplement and reduced risk of a disease or health-related condition. In this case, the evidence is not well enough established to meet the significant scientific agreement standard required for FDA to issue an authorizing regulation. Qualifying language is included as part of the claim to indicate that the evidence supporting the claim is limited (see Table 41.3). Both conventional foods and dietary supplements may use qualified health claims under this new legislation.

TABLE 41.3 Standardized Qualifying Language for Qualified Health Claims.

Scientific Ranking[a]	FDA Category	Appropriate Qualifying Language[b]
Second level	B	"... although there is scientific evidence supporting the claim, the evidence is not conclusive".
Third level	C	"Some scientific evidence suggests ... however, FDA has determined that this evidence is limited and not conclusive".
Fourth level	D	"Very limited and preliminary scientific research suggests ... FDA concludes that there is little scientific evidence supporting this claim".

[a]From Guidance for Industry and FDA: Interim Evidence-based Ranking System for Scientific Data.
[b]The language reflects wording used in qualified health claims as to which the agency has previously exercised enforcement discretion for certain dietary supplements. During this interim period, the precise language as to which the agency considers exercising enforcement discretion may vary depending on the specific circumstances of each case.

The new regulations mean that substantial agreement among scientists on whether the food or ingredient provides the health benefit claimed will not be required and food manufacturers will be able to petition the FDA to use health claims that are already approved for dietary supplements. The FDA now will use the standard of the "reasonable consumer" and "weight of evidence" to evaluate claims. The weight of the scientific evidence standard is a radical departure from the standard of evidence required for health claims under NLEA (now referred to as "A" claims).

The FDA says it believes that its new policy will enable the distribution to consumers of scientifically supported health information. As of the end of 2005, there are 14 disease-related risk reduction claims allowed in the United States as well as 14 "qualified" claims (U.S. Food & Drug Administration 2006) (Table 41.4).

41.5 EUROPEAN UNION

41.5.1 Market

For dietary supplements like functional foods, the EU represents a dichotomous market with regional differences apparent in market growth and individual product sales. According to The-infoshop (2006), the market is estimated to be worth $6.5 billion (around €5.2 billion) in 2005, the growth rate is expected to peak at 4.4% over the next year, then drop to a steady 4.1% for the subsequent five years. By 2011, the market is predicted to be worth around $8.2 billion (€6.6 billion).

The European market for functional foods in 2002 was worth about €5.7 billion, and is forecast to grow by an average of 7.5% per year, over the next five years, to reach a value of €8.2 billion by 2007, at 2002 prices and exchange rates (Euromonitor 2002).

41.5.2 Regulatory Review

Directives within the EU continue to pose problems for regulators and marketers alike. The EU adopted Directive 2002/46/EC on June 10, 2002, with the requirement that it be passed into national laws by all member states by July 31, 2003 (Hanssen 2004). It deals with laws relating to food supplements and includes only vitamins and minerals.

It should be emphasized that the obstacles to marketing functional foods are high. There are currently EU-wide initiatives to modify health claims regulation, which were expected to be complete by 2005 but have been delayed due to disagreements between the EU Parliament

TABLE 41.4 Allowed Health Claims in the United States (December 2005).

NLEA

- Calcium and osteoporosis
- Sodium and hypertension
- Dietary fat and cancer
- Saturated fatty acids, cholesterol and coronary heart disease
- Fiber-containing grain products, fruits, vegetables and cancer
- Fiber-containing grain products, fruits, vegetables and risk of coronary heart disease
- Fruits, vegetables and cancer
- Folate and neural tube defects
- Dietary sugar alcohols (sorbitol, xylitol, mannitol) and dental caries
- Dietary soluble fiber (whole oats and psyllium) and coronary heart disease
- Soy protein and coronary heart disease
- Sterols/stanols and risk of coronary heart disease

FDAMA

- Whole grains and coronary heart disease and certain cancers
- Potassium and sodium and hypertension

Qualified

CANCER

- Tomatoes and prostate, ovarian, gastric, and pancreatic cancers
- Calcium and colon/rectal cancer and calcium and colon/rectal polyps
- Green tea and risk of breast cancer and prostate cancer
- Selenium and certain cancers
- Antioxidant vitamins and risk of certain cancers

CARDIOVASCULAR DISEASE

- Monounsaturated fatty acids from olive oil and coronary heart disease

OMEGA-3 FATTY ACIDS

- Omega-3 fatty acids and reduced risk of coronary heart disease
- Walnuts and coronary heart disease
- Nuts and coronary heart disease
- Folic acid, vitamin B_6, and vitamin B_{12} and vascular disease

COGNITIVE FUNCTION

- Phosphatidylserine and cognitive dysfunction and dementia

DIABETES

- Chromium picolinate and a reduced risk of insulin resistance, type 2 diabetes

HYPERTENSION

- Calcium and hypertension, pregnancy-induced hypertension, and preeclampsia

NEURAL TUBE DEFECTS

- Folic acid and neural tube defects

and Council on health claims and notification procedures (European Commission 2005). Should EU-wide claims be approved, many industry experts believe that only large EU-based companies will be able to fulfil requirements for the scientific support for health claims that will be required and ensuring accurate translation of claims into over ten different languages.

41.6 JAPAN

41.6.1 Market

The dietary supplement market in Japan remains undeveloped in comparison with the United States, and Europe. The regulatory environment for nutritional supplements is restrictive and cumbersome (International Business Strategies 2001). Most dietary supplements are marketed through nonretail venues including the Internet and multilevel marketing firms.

Although Japan has been slow to recover from an overall economic downturn, its nutritional supplement market has been growing steadily for the last 10 years. In 1994 the market was approximately $5.0 billion, 10 years later it has doubled to $11.1 billion, an average of 11% per year growth (Yamaguchi 2005). In 2001 the market grew 22%, its largest single year growth to date. Between 2002 and 2003, growth slowed to just over a 5% increase, but in 2004, the market bounced back to double digit growth again. Despite years of economic slowdown and a difficult regulatory environment, the Japanese nutritional supplement industry showed its resilience by growing 12% in 2004 (Yamaguchi 2005).

Since the inception of FOSHU in 1991, functional food sales have been growing at an average of 25% annually. Japan is widely recognized as the most developed and established market for functional foods in the world and has the second largest functional food market behind the United States, with a value of approximately $16 billion in 2005 (Yamaguchi 2006). The Japanese spent $130 per person per year on functional foods compared with $77 per person per year in the United States, $46 for Europeans and an estimated $3 or so for other Asian countries.

Since 1990, it is estimated that over 5500 new functional foods have been introduced in Japan, an average of about 400 a year. There are 1500 to 2000 functional foods on the market and 500 of them have qualified for FOSHU status. The FOSHU market currently accounts for nearly $4.1 billion of the total health food industry in Japan, which is estimated to be worth $12 billion in 2004. In 2004, there were approximately 500 products approved by FOSHU (Yamaguchi 2006).

Currently, the "nonhealth claim" functional food market represents the largest sector of Japan's food industry at around $8 billion. This is followed by a newer category known as Foods with Nutrient Function Claims at an estimated $5 billion and including over 1000 products according to a recent Japan market report (Japan Health Food and Nutrition Food Association 2003).

41.6.2 Regulatory Review

Planning a strategy for functional food marketing in Japan is not an easy task. Food and supplement companies are faced with a number of pathways in a maze of regulations. FOSHU is one of the five categories covered under "Foods for Special Dietary Uses". Since the passage of FOSHU, the public's interest in the relationship between nutrition, diet, and life-style-related diseases and disorders has flourished.

A 2005 update to the FOSHU regulation has created three new categories of products: qualified, standardized, and disease risk reduction. Of the seven health benefit categories approved under FOSHU, 54% of products and 67% of sales are generated by the gastrointestinal health foods category. Functional pre- and probiotic drinks and yogurts dominate the market. Other ingredients approved include fiber sources; oligosaccharides; peptides and proteins; lactic acid bacteria; diacylglycerides and minerals such as calcium and iron. Smaller categories include noncariogenic sweeteners, polyphenols, chitosan, β-carotene, green tea catechins, and DHA.

41.7 THE GLOBAL BUSINESS OF FUNCTIONAL FOODS AND NUTRACEUTICALS

The global market for functional foods and ingredients is comprised of companies that see the industry as a growth opportunity and the ability to make higher margins. Although there are significant opportunities for market success, there are many challenges involved in the development and positioning of functional food products. Generally, it requires longer moving of such products into the market, primarily due to the length of time (and resources) required to prove the science behind the products. Long-term strategies require endurance, focus, and patient capital. Many investors are often not prepared to wait for financial returns that may not come to bear for 10–15 years.

Companies that have had market success to date have done so based on a clear understanding of consumer issues. For example, probiotic products have been successful in many areas of Europe because of consumer understanding of the importance of gut health. Eye health on the other hand is of great concern in both North America and Japan but to a much lesser degree in the EU.

Also of importance in building successful functional food companies is a well-established local food and food development industry that can assist with new product development and food applications for ingredients. These traditional food companies have established distribution channels in their respective domestic markets and often export markets as well.

Crucial to tapping into the potential for functional foods and supplements is establishing a market-friendly regulatory framework. Japan was the first jurisdiction globally to put in place regulatory reforms to encourage companies to utilize health claims to develop healthier foods and to communicate those messages to consumers. Part of the reason this system developed in Japan was due to that country's concern over its aging population. Food retailers and marketers are constantly looking for opportunities to directly address problems associated with aging. Europe is another leader in functional food development, benefiting from excellent public awareness of the health benefits of foods as well as a significant focus upon research. The United States is well established as another health-claim friendly environment for both food and supplement products.

41.8 CONCLUSIONS

Several trends have led to an interest in functional foods and dietary supplements by the industry, consumers, health-care professionals, and government, one of the most significant being the movement away from a prescription approach to disease treatment to a

focus upon long-term disease prevention. Functional foods and supplements will continue to grow at a strong and steady pace as consumer interest and acceptance of these products, as well as scientific substantiation of safety and efficacy, increases.

From an industry point of view, functional foods and dietary supplements offer one of the most lucrative ways to add value to existing products and to innovate with new ones. These products are being developed by companies globally in response to growing consumer demand as well as to create higher-margin, value-added products for the manufacturers and inevitably their customers and shareholders. Of extreme importance, functional foods and supplements offer the opportunity to positively impact health-care costs, which will become an increasingly significant burden to governments globally.

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42

Functional Foods Based on Dairy Ingredients

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42.1 Introduction 957
42.2 Functional Ingredients for Use in Dairy Foods 959
42.2.1 Probiotic Cultures 960
42.2.1.1 Requirements for Effective Probiotics 960
42.2.1.2 Health Benefits of Probiotic Products 961
42.2.2 Prebiotics and Synbiotics 966
42.2.3 Fiber Addition 967
42.2.4 Fortification with Minerals, Vitamins, and Health-Promoting Ingredients 968
References 969

42.1 INTRODUCTION

Dietary regime is an integral part of a healthy life-style. Recognition of the role of specific foods in diet for the prevention and control of disease and improvement of body functions is increasing. Present-day consumers are becoming more health-conscious. Accordingly, they are looking for foods associated with health benefits beyond their inherent nutritive

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value. They are motivated by their desire to control individual well-being, promote health, prevent disease, and manage certain disease states.

The current trend to provide specific health benefits beyond sustenance accorded by food intake has roots in ancient medicine. The prevention and management of disease was recognized in India some 5000 years ago with the development of Ayurvedic (Science of Life) medicine. Use of active ingredients isolated from plants, herbs, minerals, and animals formed the core of this medical practice to prevent disease and treat certain disorders. The emphasis was prevention and management of common disorders by following specific dietary patterns in response to individual body requirements. Later, in the third century B.C., Chinese emperor Shen Nong discovered that certain plants and herbs have medicinal values.

An interactive discipline of nutrition and food science has produced an array of food products that represent a vibrant and dynamic segment of the food industry. Such foods are termed as functional foods, nutraceuticals, wellness foods, or designer foods. Functional foods are characterized by their similarity with conventional foods, but when consumed as a part of the normal diet, they confer a certain health benefit to the consumer. The functional aspect may be ascribed to a naturally occurring functional component of the food or it may be made functional by incorporating functional ingredients.

Dairy products are naturally "functional" because of their calcium content and their ability to contribute specific health-promoting properties such as physiologically active proteins, peptides, conjugated linoleic acid, sphingolipids, and butyric acid (Chandan 2007). The functional attributes are further enhanced by the addition of ingredients demonstrated to possess positive physiological effects, such as probiotics, prebiotics, fiber, phytochemicals, omega-3 (ω-3) polyunsaturated fatty acids, soy proteins, antioxidants, plant sterols, lutein, chondroitin, glucosamine, and other functional ingredients (Shah 2001). In Japan, such foods are defined as foods for specified health use (FOSHU). The Ministry of Health and Welfare in Japan certifies these foods. In other countries, no formal definition of functional foods has been adopted.

The nutrition industry in the United States is of the order of $22 billion. It comprises vitamins and minerals, herbals and botanicals, sports meal and specialty, natural and organics, natural personal care, and functional foods. The functional foods comprise 37% of this industry, totaling about $8 billion. In 2003, the functional food market was 4% of the retail food sales of $555 billion and projected to grow to $45 billion in 2010 (Childs 2005).

The popularity and fast growth rate of functional foods are driven by:

1. A steep rise in health-care costs and an emphasis on prevention of disease at the government and consumer levels.
2. A rise in aging population (65 years and above), which in the United States is projected to be 33% of the total population by 2030. This segment of the population is particularly interested in healthier foods for improving health and quality of life.
3. Advances in nutritional and health sciences, food technology and biotechnology, making it possible to identify health attributes of functional ingredients and foods, and making it feasible to make them available to the consumer.
4. A widespread accessibility of health, nutritional, and medical information through the media, including the Internet.
5. Flexibility of regulatory control, facilitating marketing of functional foods.
6. A growing trend in consumers taking an active role in improving health through foods.

42.2 FUNCTIONAL INGREDIENTS FOR USE IN DAIRY FOODS

Dairy foods contain various constituents known for contributing health benefits. Among the milk constituents, whey proteins are reported to modulate the immune system, decrease hypertension, reduce the risk of cancer and heart disease, and help in nutrient transport and absorption (Hoolihan 2004). Calcium, magnesium, phosphorus, fluoride, and vitamin K in milk play a significant role in bone health and prevention of osteoporosis in later life. The ratio of calcium, phosphorus, and magnesium in milk is ideal for rapid transfer to bone mass. Calcium reduces the risk of colon cancer, formation of kidney stones, and is helpful in obesity control. Several components of milk fat possess functional value. Butyric acid has been shown to reduce the risk of colon cancer. Conjugated linoleic acid is reported to modulate the immune system and protect against cancer of stomach, colon, breast, and prostrate. Sphingolipids may reduce the risk of cancer and cardiovascular disease (Miller and others 2000). The phospholipids act as anticancer agents, antimicrobial agents against gastrointestinal pathogens, and protect against Alzheimer's disease, depression, and stress. Phosphatidylserine is recognized for reducing the risk of dementia in the elderly. The milk fat globule membrane is reported to exhibit health-promoting properties. Its components show cholesterol-lowering attributes, possess inhibitory properties against cancer cell growth, and inhibit stomach-ulcer-causing *Heliobacter pylori*. Butyrophilin, another component of the milk fat globule membrane, is a possible suppressor of multiple sclerosis. Xanthine oxidase has antibacterial characteristics. Although the functional food value of dairy ingredients is well established, it can be further enhanced.

In general, several ingredients for enhancing the functional value of foods have consumer acceptance, including oats, certain fruits including pomegranate and cranberries, vegetables including broccoli, soy, nuts, fish, whole grains, red wine, and olive oil. A number of dairy products and compounds isolated from milk fractions (whey peptides, lactoferrin, conjugated linoleic acid, galacto-oligosaccharides) are also

TABLE 42.1 Modification of Foods to make them Functional Food.

Modification	Health Benefit
Add probiotics	Enhance gastrointestinal health and immune function, lower risk of colon cancer
Add prebiotics	Enhance gastrointestinal health and immune function, lower risk of colon cancer
Add dietary fiber	Increase stool bulk and prevent constipation, reduce cholesterol and risk of heart disease, lower risk of colon cancer
Add minerals (calcium and magnesium) and vitamins	Improve nutritive value, reduce osteoporosis risk, control hypertension, and reduce risk of colon cancer
Add bioactive peptides	Control hypertension, enhance immune function, and increase bioavailability of mineral
Add omega-3 polyunsaturated fatty acids	Reduce risk of heart attack and certain cancers, enhance immune function
Incorporate phytosterols/extracts/concentrates	Provide antioxidants, lower risk of cardiovascular disease and cancer, reduce serum cholesterol and blood pressure
Add soy protein/isoflavones	Reduce risk of certain cancers and heart disease

Source: Adapted from Shah (2001).

members of the functional ingredient group. Ingredients rich in ω-3 fatty acids (docohexanoic acid and eicosapentaenoic acid), plant sterols, lutein, chondroitin, and glucosamine are popular choices for consumers seeking functional foods. Table 42.1 lists approaches to converting foods into functional foods.

In the case of dairy foods, specific ingredients of choice in the marketplace are probiotics, prebiotics, fiber, soy protein, plant sterols, and fortification with vitamins, calcium, and magnesium.

42.2.1 Probiotic Cultures

Probiotics are defined as a food or supplement containing defined strains of living microorganisms that on ingestion in certain doses exert health benefits beyond their inherent basic nutrition. They are believed to contribute to the well-being of the consumer by improving the host's microbial balance in the gastrointestinal tract (Chandan 1989, 1999; Shah 2001; Gilliland 2003). This definition stresses the importance of ingestion of several hundred millions of live and active microbial cultures. Recent advances in probiotic research show much promise in new product development of functional foods based on milk. There has been marked proliferation in the number of probiotic products on the market. Milk is an excellent medium to carry or generate live and active cultured dairy products. Probiotics and associated ingredients add an attractive dimension to cultured dairy foods for effecting special functional attributes (Sanders 1994; Chandan 1999; Schaafsma and Steijns 2000; Mattila-Sandholm and Saarela 2003).

42.2.1.1 Requirements for Effective Probiotics. Criteria for live and active cultures have been established by the industry with a view to maintain the integrity of refrigerated and frozen yogurts. The probiotic organisms must implant and multiply in the gut to avoid them from being expunged entirely. They must not only be able to tolerate and pass through the high acidic condition of the stomach, but also be able to grow and proliferate at physiological levels of bile salts and adhere to the intestinal epithelial cells. Bile salts are essential in helping to emulsify fat before it can be digested in the intestine. The probiotics that can colonize should also be resistant to several antibiotics and a good producer of natural selective antimicrobial bacteriocins. A strain of *Bifidobacterium* should be negative for production of catalase, nitrate reductase, urease, and for the formation of indole. In addition, liquefaction of gelatin, gas formation from glucose, response to rhamnose, sorbose, glycerol, erythritol, adinotol, and dulcitol should be negative. Commercial probiotic strains must have verified safety of use in the human diet. They should possess stability to acid and bile, exhibit colonization and adherence in the gastrointestinal tract, and should be able to elaborate a bacteriocin (Salminen and others 1998).

There are 56 species of *Lactobacillus* and 29 species of *Bifidobacterium*. Probiotic cultures that provide potential health benefits are shown in Table 42.2. The probiotics constitute organisms from mainly the genera *Lactobacillus*, *Enterobacter*, *Streptococcus*, and *Bifidobacterium*. These genera have been shown to be important members of the gastrointestinal microflora and are all relatively beneficial. The strains of lactic acid bacteria used in probiotics are mostly intestinal isolates such as *L. acidophilus*, *L. casei*, *Enterococcus faecium*, and *Bifidobacterium bifidum*. All probiotics, whether intestinal

TABLE 42.2 Probiotic and Beneficial Microorganisms in Commercial Products.

Lactobacillus Species and Strains	*Bifidobacterium* and Strains	Other Organisms
L. acidophilus La2, La5/La2, NCFM, DDS-1, SBT-2062, and LAFTI L10	*B. adolescentis*	*Enterococcus faecium*
L. casei, Shirota, Immunitass, 744, 01	*B. bifidum*, Bb-11 and BB 12	*Pediococcus acidilactici*
L. crispatus	*B. breve*, Yakult	*Saccharomyces boulardii*
L. delbrueckii subsp. *bulgaricus*, Lb12	*B. essensis*, Danone (BioActive)	*Streptococcus thermophilus*
L. fermentum, RC-14	*B. infantis*, Shirota, Immunitass, 744 and 01	
L. gasseri	*B. lactis*, Bb-02, LAFTI B94 and DR 10.	
L. helveticus, BO2	*B. laterosporus*, CRL 431.	
L. johnsonii La1/Lj1	*B. longum*, B536 and SBT-2928	
L. lactis, La1	*B. subtilus*	
L. paracasei, CRL 431 and F19		
L. plantarum, 299v and Lp01		
L. reuteri, SD2112/MM2		
L. rhamnosus, GG, GR-1, 271, LB21 and DR20.		
L. salivarius		

Source: Adapted from Chandan (1999), Shah (2001, 2004), Saxelin and others (2004).

isolates or not, require regular consumption in adequate doses; it should not be assumed that a few doses will allow the organisms to colonize the gut permanently.

L. acidophilus and *Bifidobacterium* spp. are known to differ widely in their ability to grow in the presence of bile salts (Shah 2001). Both are reported to be stable at various concentrations of bile salts. Yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) are included in the list of probiotic cultures because they possess a distinctly high lactase activity, making yogurt easily digestible by individuals with a lactose-maldigestion condition. To bolster probiotic function, most commercial yogurt is now supplemented with *L. acidophilus*, *Bifidobacterium* spp., and *L. casei*. Unfermented milk, ice-cream, and cheese are also being used as carriers of probiotics.

42.2.1.2 Health Benefits of Probiotic Products. The belief in the beneficial effects of the probiotic approach is based on the knowledge that the intestinal microflora provides protection against various diseases. Probiotics have been with us for as long as people have eaten fermented milk. It can be shown that germ-free animals are more susceptible to disease than their conventional counterparts that carry a complete gut flora. This difference has been shown for infections caused by *Salmonella enteritidis* and *Clostridium botulinum*. Another source of evidence that supports the protective effect of the gut flora is the finding that antibiotic-treated animals, including humans, can become more susceptible to disease. Probiotics also deplete the essential nutrients for the pathogenic organisms, thus eliminating their growth. Table 42.3 shows some of the potential health benefits of probiotic cultures.

TABLE 42.3 Potential Health Benefits Related to Probiotics.

Health Condition	Benefit
Gastrointestinal disorders	The balance of various types of intestinal bacteria is disturbed by stress, antibiotic therapy, and age, leading to proliferation of pathogenic organisms and suppression of friendly bacteria, causing clinical disorders, namely, infections, inflammatory diseases, and immune deficiency. Probiotics restore intestinal microbial balance leading to general well-being and intestinal health
Lactose intolerance	Intake of yogurt and some probiotics improve lactose digestion and alleviate lactose intolerance condition.
Diarrhea	Strain-dependent probiotics prevent and treat diarrhea related to rotavirus infection, antibiotic therapy, and travelers' diarrhea
Immune-system-related diseases	Enhanced immune function resulting from stimulation of certain cellular and antibody functions may increase resistance to infections, gastrointestinal disturbances, allergies, and cancer
Infectious diseases	Probiotics may help prevent or treat respiratory infections, postoperative infections and control *Helicobacter pylori*, which is involved in type-B gastritis, ulcers, and possible cancer of the stomach. Possible control of food-borne diseases such as salmonella and *E. coli*
Colon cancer	Intake of yogurt and certain probiotics may reduce the development of precancerous lesions and chemically induced tumors. Enzyme biomarkers of colon cancer are inhibited by specific dairy cultures
Allergy	Probiotics may help in preventing allergic reactions caused by food allergens. They may also prevent recurring atopic eczema in children
Irritable bowel syndrome	Probiotics may reduce abnormal bloating symptoms of diarrhea-predominant IBS
Inflammatory bowel disease	Probiotics influence intestinal microflora and may reduce symptoms for individuals with ulcerative colitis, Crohn's disease, and pouchitis
Coronary heart disease	Certain probiotic strains may reduce serum cholesterol and glycerides and blood pressure, thereby reducing the risk of heart disease
Miscellaneous disorders	Certain probiotics may prevent and treat urinary tract infections, bacterial vaginosis, and yeast vaginitis in women and reduce colic in infants. Probiotics may protect from dental caries, prevent constipation, reduce inflammation of arthritis, and formation of kidney stones

Source: Adapted from Chandan (1999), Shah (2001), Dairy Council Digest (2005).

Balancing Intestinal Microflora. More than 400 different species of bacteria inhabit the human gastrointestinal tract. Their collective diverse population exceeds the total cell counts of the human body. Most of the bacteria do not cause any disease, but some of them do have the potential to cause disease if their level exceeds a critical level. As a result of aging, enhanced stress level in life, antibiotic therapy, and pollution, the balance in intestinal bacteria is disrupted in favor of potentially pathogenic bacteria. The loss of balance leads to increased risk of clinical disorders such as infectious diseases,

inflammatory illnesses, and unfavorable changes in the immune system. Research has shown that probiotic intake helps maintain proper and healthy balance of microbial population in the intestines, conferring upon the host general well-being and several therapeutic benefits (Dairy Council Digest 2005).

Improvement in Lactose Digestion. Other beneficial effects of probiotics include improvement of lactose utilization by the large proportion of the world's population unable to effectively digest lactose. The enzyme responsible for lactose digestion, lactase, although present in the suckling infant, disappears after weaning. Lactose malabsorption refers to incomplete digestion of lactose resulting in a flat or low rise in blood sugar following ingesting lactose in a clinical lactose intolerance test. Normally, the disaccharide lactose is hydrolyzed to glucose and galactose by lactase, and is subsequently absorbed in the small intestine. Lactase is a constitutive, membrane-bound enzyme located in the brush borders of the epithelial cells of the small intestine. In individuals with impaired lactase activity, lactose is not fully metabolized and the intact residual lactose enters the colon where it is fermented by inherent microflora to generate organic acids, carbon dioxide, methane, and hydrogen. The fermentation products, together with the osmotically driven excessive water drawn into the colon, are primarily responsible for abdominal pain, bloating, cramps, loss of appetite, diarrhea, and flatulence. These symptoms associated with lactose maldigestion are experienced when lactose is not fully digested in the small intestine. It is well established now that lactose-deficient subjects tolerate lactose in yogurt better than the same amount of lactose in milk. It has been conclusively demonstrated that intake of yogurt and certain probiotics alleviates symptoms of lactose intolerance. The lactase contained in the yogurt culture is mainly responsible for lactose digestion in the gut lumen. The bacterial cells of the yogurt culture undergo lysis, releasing the enzyme lactase into the small intestine. An increase in lactase activity in the small intestine of humans on yogurt has been shown. The microbial origin of the lactase is shown by the large increase in activity in the gut contents compared with the gut wall.

Prevention of Diarrhea. Probiotics are reported to improve the efficiency of the digestive tract, especially when bowel function is poor. Establishment of probiotics in the gastrointestinal tract may provide prophylactic and therapeutic benefits against intestinal infections. Probiotics may also have role in circumventing traveler's diarrhea (Fernendes and others 1992; Elmer and others 1996). Yogurt and probiotic cultures of *L. rhamnosus* GG as well as a blend of *L. acidophilus*, *B. bifidum*, *L. bulgaricus*, and *S. thermophilus* have been reported to reduce the incidence of traveler's diarrhea.

Yogurt containing probiotics has been found to reduce the duration of diarrhea caused by rotavirus in infants and young children. *L. rhamnosus* GG and a combination of *L. reuteri* DSM 12246 and *L. rhamnosus* 190702 have been found very effective in this regard. Fermented milk with probiotics has been recommended to replace milk during diarrhea treatment because it is tolerated better than milk. A double blind study has shown that only 7% of infants receiving probiotic formula containing *B. bifidum* and *S. thermophilus* developed diarrhea, composed with 31% incidence in the placebo group (Saavedra and others 1994).

Another type of diarrhea is related to antibiotic therapy. The growth of *C. difficile* and other pathogenic organisms are the main causative agents. Clinical trials have shown that yogurt containing live cultures of *L. rhamnosus* GG, *L. acidophilus* LA1, as well as

Saccharomyces boulardi are effective in reducing the incidence or severity of antibiotic-induced diarrhea.

Stimulation of the Immune System. An interesting development in recent years has been the finding that lactobacilli and bifidobacteria administered through the mouth can stimulate macrophage activity against several different species of bacteria (Brassart and Schiffrin 1997; Rangavajhyala and others 1997). For example, *L. casei* given to mice increased phagocytic activity. Lactobacilli injected intravenously survived in the liver, spleen, and lungs, and enhance the natural killer cell activity.

Yogurt, certain lactobacilli, and bifidobacteria enhance cellular and antibody functions of the immune system, resulting in an increase of resistance to infections, cancer, allergies, and gastrointestinal disorders (Dairy Council Digest 2005). In human trials, secretory immunoglobulin A (involved in protection against microbial antigens at the intestinal mucosal surface) was shown to increase as a result of feeding milk fermented with *L. acidophilus.* Macrophages constitute the first line of defense against bacterial invasion. In mice, it was reported that feeding milk fermented individually, or in combination with *L. casei* and *L. acidophilus*, led to a marked rise in macrophages. Cytokines have diverse functions in the immune system and their production is enhanced by feeding milk fermented with probiotics including *L. casei* and *L. rhamnosus* GG. Healthy volunteers given yogurt daily have been shown to markedly increase their gamma interferon level. In elders with poor functioning immune systems, *B. lactis* HNO19 increased their cellular immunity factors, such as total, helper, and activated T-cells, tumor-killing cells, and leukocytes phagocytosis. Studies with *L. rhamnosus* GG have shown that a cellular response to intestinal organisms is enhanced in healthy adults. The probiotic organisms aided in balancing anti-inflammatory and pro-inflammatory cytokines. The probiotic organisms increased the response of peripheral T-lymphocytes to intestinal bacteria. Also, an increase in anti-inflammatory response was observed, possibly due to an increase in the secretion of suppressive cytokines and a decrease in the secretion of pro-inflammatory cytokines.

Prevention of Infectious Diseases and Elaboration of Bacteriocins. Probiotics may be helpful in preventing and treating respiratory infections and postoperative infections. In this regard, *L. rhamnosus* GG has been clinically shown to reduce the incidence and severity of respiratory infections in children. Ingestion of fermented milk containing a mixture of *L. rhamnosus* GG, *Bifidobacterium* spp., *L. acidophilus*, and *S. thenmophilus* has been clinically shown to reduce potentially pathogenic bacteria in the upper respiratory tract of humans (Dairy Council Digest 2005).

Several studies have been reported on the effect of probiotics on *H. pylori*, which causes ulcers of the stomach and B-type gastritis. The results show that *L. casei* Shirota, *Bifidobacterium*, and *L. salivarius* are inhibitory to the growth of *H. pylori*. In human adults with *H. pylori* infection, it was shown that yogurt containing *L. johnsonii* suppressed the growth of *H. pylori* and reduced gastric inflammation.

Probiotics actively produce antibacterial substances called bacteriocins, which kill or inhibit pathogenic bacteria. The bacteriocin acidophilin, produced by *L. acidophilus*, has been shown to kill 50% of 27 different pathogenic bacteria studied. Children with Salmonella poisoning and Shigella infections were cleared of all symptoms using *L. acidophilus*. *Bifidobacterium bifidum* effectively kills or controls *E. coli*, *S. aureus*, and *Shigella* (Fernandes and others 1992).

Anticarcinogenesis. Bifidobacteria, and lactobacilli, and especially *L. acidophilus* have been shown to have anticarcinogenic properties against certain tumors (Goldin and Gorbach 1992). Several reports suggest prevention of cancer initiation by various probiotics. Probiotics have been shown to reduce biomarkers (fecal procarcinogenic enzymes nitroreductase and azoreductase) of colon cancer risk. An epidemiological study reported a positive correlation between probiotic consumption and prevention of colon cancer (Fernandes and others 1992). Live cells of probiotics exhibit inhibition of a large variety of mutagens (Shah 2001).

Reduction in Serum Cholesterol. Elevated levels of serum cholesterol, particularly LDL-cholesterol, have been linked to an increased risk for cardiovascular disease. Feeding of fermented milks containing very large numbers of probiotic bacteria ($\sim 10^9$ bacteria/g) to hypercholesterolemic human subjects has resulted in lowering cholesterol from 3.0 to 1.5 g/L. The role of bifidobacteria in reducing serum cholesterol is not completely understood. Mann and Spoerry (1974) observed a decrease in serum cholesterol levels in men fed large quantities of milk fermented with *Lactobacillus*, possibly due to the production of hydroxymethyl-glutarate by lactic acid bacteria, which inhibits hydroxymethylglutaryl-CoA reductases required for the synthesis of cholesterol.

Klaver and Meer (1993) reported that removal of cholesterol from the culture medium by *L. acidophilus* and other species is not due to bacterial uptake of cholesterol, but results from bacterial bile salt deconjugating activity. The deconjugated bile acid does not absorb lipid as readily as its conjugated counterpart, leading to a reduction in cholesterol level.

Reports by Gilliland and others (1985) showed that *L. acidophilus* itself may take up cholesterol during growth in the small intestine and make it unavailable for absorption into the blood stream. Probiotics effectively help to reduce serum cholesterol levels. Some studies have indicated a modest lowering of serum cholesterol in subjects consuming milk fermented with *L. acidophilus*, *L. rhamnosus* GG, and yogurt cultures (Gilliland 2003).

Anti-inflammatory Effects. Inflammatory bowel diseases (IBD, ulcerative colitis, and Crohn's disease) are related to the intestinal microflora. IBD affects up to two million people worldwide. Symptoms include a disturbance in bowel habits and mucosal inflammation. In the case of IBD, the number of *Lactobacillus* and *Bifidobacterium* in the gastrointestinal tract is lower, and that of coccoids and anaerobes higher. Probiotics are not reported to cure the disease. However, once patients are in remission through treatment with corticosteroids, some probiotics can prolong the remission, thus reducing the relapses and the use of corticosteroids. This improves the quality of life of patients. Several reports have suggested that probiotics reduce symptoms and prevent relapse in patients suffering from ulcerative colitis, Crohn's disease, and pouchitis. Also, probiotics are reported to reduce abnormal bloating, a symptom of irritable bowel syndrome.

Urinary Tract Infections, Vaginitis, and Allergy. The vaginal microflora changes drastically during bacterial infection. Bacteria of genera Escherichia, Proteus, Klebsiella, and Pseudomonas along with the yeast *Candida albicans* are recognized as etiological agents in urinary tract infections among adult women. Normal urethral, vaginal, and cervical flora of healthy females can competitively block the attachment of uropathogenic bacteria to the

surfaces of uroepithelial cells. Lactobacilli strains supplemented in the diet or directly applied will coat the uroepithelial wall and prevent the adherence of uropathogens. Milk fermented with yogurt cultures and *L. casei* influences the intestinal microflora of infants (Guerrin-Danan and others 1998).

Therapeutically, probiotics have been reported to be useful in the treatment of acne, psoriasis, eczema, allergies, migraine, gout, rheumatic and arthritic conditions, cystitis, and candiadiasis.

Potential mechanisms by which probiotics may exert their beneficial effects are:

1. Competition with other microflora for nutrients;
2. Production of acids inhibitory to certain enteropathogens;
3. Production of bacteriocins or inhibitory metabolites;
4. Immunomodulation; and
5. Competition for adhesion to intestinal mucosa.

Future research work with well-designed human studies is needed to make a definitive validation of many health-benefit claims. Several benefits are specifically related to species and strain of the probiotic. Accordingly, identification of beneficial strains in each species is critical in probiotic research. As efficacy of a probiotic is directly related to the number of live and active culture cells consumed, it is important to specify potency or colony-forming units (cfu) of the culture per unit weight or volume of the product. Additionally, the culture should be active in terms of growth potential (Chandan 1997; Chandan and others 1999).

42.2.2 Prebiotics and Synbiotics

The term "prebiotic" refers to a variety of oligosaccharides and other food ingredients, which, on ingestion, promote the growth of beneficial microorganisms in the gastrointestinal tract. Prebiotics remain unaltered by digestive juices and enter the colon intact. In the colon, they are fermented to produce short-chain fatty acids, thereby reducing the pH of the colonic contents. This effect leads to inhibition of the pathogenic inhabitants of the colon and changes the balance in favor of beneficial organisms. Chicory and Jerusalem artichokes are major sources of prebiotics. They are also found in honey and onions. These prebiotics include fructans, inulin, and oligosaccharides. Both fructooligosaccharides (FOS) and galacto-oligosaccharides are widely used as prebiotics. In addition, some lactose derivatives such as lactulose, lactitol, and lactosucrose also function as prebiotics. Starch-based prebiotics include malto- and isomalto-oligosaccharides, polydextrose, and certain resistant starches. Some gums can also act as prebiotics. Supplementation of probiotics with prebiotics can be a very effective functional food. For example, fructooligosaccharide (FOS) is exclusively utilized by a few strains of *Bifidobacterium bifidum* and *L. acidophilus*. Thus, a combination of FOS along with these cultures will induce the proliferation of these cultures in preference to other microflora (Shah 2004). This synergistic combination is termed as synbiotics. This has also been confirmed in human volunteers where FOS consumption increased the levels of bifidobacteria at the expense of less desirable bacterial species. The health benefits of prebiotics are similar to those associated with probiotics. Additionally, FOS supplements have been

shown to substantially improve the absorption of calcium and magnesium, thereby helping improve bone health.

42.2.3 Fiber Addition

The daily values for dietary fiber are 25 and 30 g for a 2000- and 2500-calorie diet, respectively. The main sources of dietary fiber are fruits, vegetables, and whole grains. As dairy products are naturally deficient in fiber, these ingredients should be of much interest in enhancing their functional value. For fortification, an array of dietary fibers is available to choose from. However, for successful product development, the fiber must not alter the perceived sensory properties of the final product.

Apart from the laxation benefit, fiber consumption lowers cholesterol, slows absorption of nutrients into the bloodstream, and promotes healthy gut ecology. Accordingly, dietary fiber confers a positive influence on human health and longevity. Epidemiological, experimental, and clinical research has shown that consumption of diets high in fiber lowers the risk of obesity, colorectal cancer, cardiovascular disease, and possibly type-2 diabetes. For example, a diet containing viscous polysaccharides lowers the low-density lipoproteins (LDL) and total serum cholesterol by bile acid turnover and lipid absorption. Furthermore, the beneficial effect may be ascribed to slow down of the absorption of carbohydrate, increase in stool bulk, and production of short-chain fatty acids in the colon.

Dietary fiber is classified as soluble fiber and insoluble fiber. In general, soluble fiber is beneficial in terms of cardiovascular disease and the insoluble fiber in the prevention of colon cancer. Cellulose and its derivatives are excellent insoluble fibers. Hydrocolloids furnish at least 85% soluble (on a dry weight basis) dietary fiber. Total dietary fiber content of gum acacia and gum tragacanth ranges from 80–90%, whereas their soluble fiber content is 100%. Guar gum has 90% total dietary fiber, but it is 100% soluble fiber. Pectin is 90–100% total dietary fiber and soluble gum. Oat bran is 22% total dietary fiber, 50% soluble fiber. The insoluble dietary fiber is furnished by wheat bran (45–55% total dietary fiber, 2–6% soluble fiber), rice bran (75% total dietary fiber, 4% soluble fiber), oat fiber (85–90% total fiber, 1–3% soluble fiber), and barley bran (50–70% total dietary fiber, 3–9% soluble fiber). Other sources of fibers are cottonseed, soy, and citrus. Even the fructooligosaccharides primarily used as prebiotics act like soluble fiber. Partially hydrolyzed guar gum provides excellent solubility and clarity while maintaining the health-promoting properties of a soluble gum.

Soluble fibers such as pectin and gums form gels in the stomach, thereby slowing the absorption of certain nutrients through the small intestine. Foods containing soluble fiber such as β-glucan are permitted by FDA to make a specific coronary heart disease claim. For example, 0.75 g of soluble fiber from oat ingredients or 1.7 g of soluble fiber from psyllium husk can make such a claim on the package. Insoluble fibers such as polysaccharides (cellulose, hemicellulose), oligosaccharides, and lignin pass through the digestive tract intact until they reach the colon, where they exert positive physiological effects like laxation, attenuation of serum cholesterol, and blood glucose.

Dietary fiber (psyllium, guar gum, gum acacia, oat fiber, soy components) as well as multivitamin–mineral mixes are being incorporated in fat-free milk to provide targeted

niche consumers meal replacements. Such products supply a substantial proportion of daily essential nutrients.

42.2.4 Fortification with Minerals, Vitamins, and Health-Promoting Ingredients

A balance of vitamins and minerals is necessary for optimal health and well-being. Accordingly, a strategy to provide optimal daily requirements of major vitamins and minerals per serving is popular in the market. Traditionally, milk has been fortified with vitamins A and D. Now, popular ingredients of functional significance are being incorporated to enhance market value of dairy foods and dairy-based foods. Some of these ingredients designed to enhance consumer appeal are

1. Calcium, claimed to prevent osteoporosis and cancer, and to control hypertension;
2. Antioxidants (vitamins C and E), claimed to communicate the benefit of prevention of cancer, cardiovascular disease, and cataracts.

Further, it is now known that factors in diet influencing reduction in cardiovascular disease are generally constituents of plant foods: antioxidants, phenolics, carotenoids, and flavonoids. The phytoestrogens (namely, coumesterol) of certain beans may reduce bone loss. Isoflavones present in soy are recognized to be beneficial in reducing the risk of cancer and heart disease. Soy-protein-fortified yogurt is being marketed to enhance its functional value. Plant sterols clinically proven to reduce serum cholesterol levels are available and are being used as an ingredient in "heart healthy" yogurt.

Folic acid is now considered to be significant for its role in preventing heart disease. Folic acid is also significant in lowering the risk of neural tube defect in babies. Thus, new diet strategies for optimum health involve consumption of a much higher level of fruits, vegetables, legumes, nuts, and whole grains than previously believed. Breakfast yogurt containing whole grains and nuts along with vitamin fortification is another commercial approach to increasing the functional food status of yogurt.

Phosphatidylserine is a part of phospholipids. It is heavily concentrated in the cell membranes of the brain. It provides structural support and keeps the cells fluid and flexible. Scientific evidence for reduction of risk between phosphatidylserine and dementia in the elderly is recognized. Accordingly, this ingredient could be used for prevention of cognitive dysfunction in the elderly. The FDA has allowed a qualified health claim on the label "Consumption of phosphatidylserine may reduce the risk of dementia in the elderly" or "Consumption of phosphatidylserine may reduce the risk of cognitive dysfunction in the elderly".

In addition to the infant formula line of products based on fat-free milk, there is a proliferation of energy and weight reduction shake drinks for consumer segments ranging from adults to geriatric populations.

Certain successful ideas from the food industry may have a merit for application to dairy products. Antioxidants have shown promise, but a fortification strategy must include an understanding of their impact on flavor, texture, mouthfeel, and shelf-life of the product. Also, it is imperative to know a meaningful dose–benefit relationship associated with the specific fortified dairy food.

Functional fatty acids belong to a class of ω-3 fatty acids. They provide benefit to infants, children, adults, and the elderly alike. The U.S. FDA has allowed the qualified claim "Limited evidence suggests an association between consumption of fatty acids in

TABLE 42.4 Various Milk-Based Product Categories Containing Milk Fractions.

Category	Food or Supplement
Clinical nutrition	Total enteral formulas containing casein, milk proteins, and their hydrolyzates for tube or oral administration in hospitalized patients
Seniors nutrition	Whey-protein-based drinks promote bone health, provide a good source of biologically active proteins and peptides, rapid digestion rate, and induce high postprandial protein synthesis and balance. Provide essential amino acids, branched-chain amino acids and high cysteine levels for maintenance of glutathione status; improve immune system
Sports nutrition	Drinks, tablets, energy bars, or cookies can deliver bioavailable calcium, easily absorbable protein hydrolyzates, and branched-chain amino acids, antioxidant sulfur- rich amino acids, bioactive peptides and bioavailable milk minerals; glutamine peptide supports immune system and facilitates iron absorption; lowers body fat and builds muscle
Infant nutrition	Demineralized whey, lactose, caseinates, milk protein concentrate, and dairy minerals are constituents of hypoantigenic and hypoallergenic humanized infant formulas
Weight-reduction drinks	Meal replacement milk shakes fortified with such supplements as milk proteins, vitamins and minerals, and prebiotics

Source: Adapted from Chandan (1999).

fish and reduced risks of mortality from cardiovascular disease for general population". The functional fatty acids are docohexanoic acids (DHA) and eicosapentaenoic acids (EPA), which are long-chain polyunsaturated fatty acids, found in fish oil, but which can be derived from marine algae and plant sources like safflower oil. Other polyunsaturated (α-linolenic) and mono-unsaturated fatty acids (oleic) are also beneficial. Epidemiological studies have suggested that individuals at risk for coronary heart disease benefit from the consumption of plant- and marine-derived ω-3 fatty acids. These fatty acids are claimed to inhibit cancer, anti-allergy effects, and improvement in learning ability. They are involved in brain and eye development in infants. Commercially available milk fractions, especially whey proteins and other fractions, are being used in a variety of milk-based products (Table 42.4).

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43

Functional Properties of Milk Constituents

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43.1 Introduction 971
43.2 Chemical Composition of Milk 972
43.3 Nutritional Characteristics of Milk Constituents 972
43.4 Functional Properties of Milk 973
43.4.1 Milk Proteins 973
43.4.1.1 Casein 974
43.4.1.2 Whey/Serum Proteins 977
43.4.2 Milk Fat 979
43.4.2.1 Physiological Effects of Milk Fat Components 979
43.4.3 Lactose 982
43.4.4 Minerals 982
43.4.4.1 Functional Role of Calcium and Other Minerals 982
43.4.5 Vitamins and Some Other Minor Constituents 984
43.4.6 Enhancement of Functional Properties by Culturing of Milk 984
43.4.6.1 Probiotics 985
43.4.6.2 Beneficial Microflora 985
References 986

43.1 INTRODUCTION

This chapter provides basic information relative to milk constituents possessing a physiological role in addition to their nutritional contribution.

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

TABLE 43.1 Composition of Milk Commonly Used for Consumption in Various Regions of the World.

Animal	(%) Water	(%) Protein	(%) Fat	(%) Lactose	(%) Ash
Cow	87.3	3.4	3.7	4.8	0.7
Buffalo	82.7	3.6	7.4	5.5	0.8
Goat	87.7	2.9	4.9	4.1	0.8
Sheep	80.7	4.5	7.4	4.8	1.0
Horse	88.8	2.5	1.9	6.2	0.5
Camel	86.5	3.1	4.0	5.6	0.8

Source: Adapted from Fox (2003), Aneja and others (2002).

From the chemical standpoint, milk is a complex fluid in which more than 100,000 separate molecules and chemical entities have been found. In the United States, cow's milk for commercial purposes is the whole, clean lacteal secretion of one or more healthy cows properly fed and kept, excluding that obtained within 15 days before calving and 3–5 days after. This would exclude colostrum, the milk secreted immediately after birth of the calf. Colostrum is an established functional food containing high levels of antibodies for protection of the new-born calf.

It is necessary to understand the chemical, physical, and nutritional composition of milk to comprehend the functional aspects of its constituents.

43.2 CHEMICAL COMPOSITION OF MILK

In the food industry, the composition of milk is generally described in terms of its commercially important constituents, milk fat and nonfat Solids (NFS) or milk solids-not-fat (MSNF). The MSNF consists of protein, lactose, and minerals. As per FDA standards of identity, milk is "the lacteal secretion practically free of colostrum, obtained by complete milking of one or more healthy cows, which contains not less than 8.25% MSNF and not less than 3.25% milk fat". The major constituents of milk are given in Table 43.1.

43.3 NUTRITIONAL CHARACTERISTICS OF MILK CONSTITUENTS

Milk is composed of a unique set of a constituents. Milk has been described as nature's nearly perfect food, as it provides vital nutrients including proteins, essential fatty acids, minerals, and lactose in balanced proportions. Leading nutrition experts recognize milk and milk products as important constituents of a well-balanced and nutritionally adequate diet. In this regard, milk products complement and supplement nutrients available from grains, legumes, vegetables, fruits, meat, seafood, and poultry.

TABLE 43.2 Physical State and Particle Size Distribution in Milk Facilitating Nutritional and Physiological Effects.

Physical State	Type of Particles	Size, Diameter (nm)
Emulsion	Fat globules	2000–6000
Colloidal dispersion	Casein–calcium phosphate	50–300
	Whey proteins	4–6
True solution	Lactose, salts, and other substances	0.5

In terms of physical structure (Table 43.2), milk is an opaque, white, heterogeneous fluid in which various constituents are held in multidispersed phases of emulsion, colloidal suspension, or solution. The phase of the milk constituents influences the physiological characteristics of the milk constituents.

43.4 FUNCTIONAL PROPERTIES OF MILK

Various milk constituents contributing physiological effects are proteins, fat, lactose, minerals, and cultures in fermented milk products. Milk proteins have a high nutritional value because of their high essential amino acid content. Thus, they complement and balance the amino acid composition of the relatively lower quality of several vegetable proteins in the human diet. All the dairy constituents perform a nutritional function as well as physiological functions. They act independently and synergistically with each other. The role of major and minor constituents in human nutrition is intertwined with newly discovered physiological benefits.

We will now consider functional aspects of major milk constituents in more detail.

43.4.1 Milk Proteins

The major proteins of milk are casein and whey proteins in the ratio of 80 : 20. Casein further consists of various fractions including α_{S1}- and α_{S2}-casein, β-casein, and κ-casein (Table 43.3). Also shown are the major whey proteins of milk.

A number of proteins and peptides derived from milk proteins have physiological activity:

1. Immunoglobulins, lactoperoxidase, lactoferrin, and folate-binding protein;
2. Insulin-like growth factors (IGF-1 and IGF-2), mammary-derived growth factors MDGF-I and MDGF-II, transforming growth factors ($TGF_{\alpha 1}$, $TGF_{\alpha 2}$, TGF_{β}), fibroblast growth factors, platelet-derived growth factors, bombesin, and bifidus factor;

TABLE 43.3 Proteins of Cow's Milk and Their Fractions.

	Concentration (g/L)
Casein Fractions	
α_{s1}-Casein	10.3
α_{s2}-Casein	2.7
β-Casein	9.7
κ-Casein	3.5
C-terminal β-casein fragments	0.8
Whey Protein Fractions	
N-terminal β-casein fragments	0.8
β-Lactoglobulin	3.4
α-Lactalbumin	1.3
Immunoglobulins	0.8
Bovine serum albumin	0.4

Source: Adapted from Chandan (1999), Schaafsma and Steijns (2000).

3. Peptides derived from milk proteins, such as glycomacropeptides from κ-casein, phosphopeptides from caseins, caseinomorphins, immunomodulating peptides, platelet-modifying peptide, angiotensin-converting enzyme (ACE) inhibitor (which lowers blood pressure), calmodulin-binding peptides, and bactericidal peptides from lactotransferrin (Otter 2003).

Both caseins and whey proteins of milk possess physiological and biological properties. The biological properties of milk proteins are summarized in Table 43.4.

43.4.1.1 Casein. Casein proteins are further divided into α_{s1}-, α_{s2}-, β-, and κ-fractions, which along with whey proteins, β-lactoglobulin and α-lactalbumin, are gene-derived proteins synthesized in the mammary gland. All these proteins are heterogeneous and exhibit genetic polymorphs. There are between 2 and 8 genetic variants differing from each other in 1 to 14 amino acids. The variants may have an impact on protein concentration and the functional properties of milk. The γ-fraction originates from β-casein by the effect of the native proteolytic enzyme of milk.

Caseins display distinctive structure, charge, physical, and biological properties, as well as a nutritional role. The interaction of various caseins and calcium phosphate contributes to the formation of large colloidal complex particles called casein micelles. Hydrophobic interactions with calcium phosphate and submicelles seem to be involved in the formation of micelles. Micelle composition consists of 63% moisture and the dry matter consists of 92–94% protein and 6–8% colloidal calcium phosphate. Other associated salts are magnesium and citrate.

The caseins are phosphorylated proteins, containing 1–13 phosphoserine residues. κ-Casein exists in as many as nine glycosylated forms. It contains two cysteine

TABLE 43.4 Functional Attributes of Milk Proteins.

Protein	Physiological Effect
Caseins	Precursors of bioactive peptides; calcium and phosphorus carrier
Whey proteins	Confer passive immunity for disease prevention; reduce risk of heart disease and lower blood pressure, antiviral and anticancer activity, control of gut microflora, control of cellular glutathione level
β-Lactoglobulin	Binds zinc, calcium, and fat-soluble vitamins. The branched-chain amino acids enhance the immune system
α-Lactalbumin	Lactose synthesis in mammary gland, anticarcinogenic and immune-enhancing effects. May be associated with stress reduction, increase serotonin production in the brain, improve mood, and decrease cortisol level
Immunoglobulins A, M, E, G	Antibodies against diarrhea and GI tract disturbances. Support passive immune function
Bovine serum albumin	Antioxidant and antimutagenic. Binds free fatty acids and pro-oxidant transition metals
Lactoferrin	Bacterial antitoxin binding, antibacterial, antiviral, immune modulating, antinflammatory, antithrombic activity, anticarcinogenic, antioxidant, iron absorption
Lactoperoxidase	Antimicrobial, antioxidant, immune-enhancing properties
Lysozyme	Antimicrobial, synergistic with immunoglobulins and lactoferrin

Source: Adapted from Chandan (1999), Harper (2000), Hoolihan (2004), Kreider (2004).

molecules per molecule. As a result of disulfide bond formation, it can exist as polymers of 2–8 units. Similarly, α_{s2}-casein also contains two cysteines and exists in a dimeric form.

Casein micelles contain α_{s1}-, α_{s2}-, β-, and κ-casein in the ratio of 3 : 1 : 3 : 1. Most of the fractions α_{s1}-, α_{s2}-, and β-casein are located in the interior of micelles, with κ-casein predominantly wrapped around the surface of the micelle. Casein fractions in the interior of the micelles are sensitive to calcium and become insoluble in the presence of calcium. However, κ-casein is not sensitive to calcium and thereby keeps the micelles containing calcium-sensitive caseins intact and suspended in the aqueous phase. κ-Casein is a protein with a hydrophilic carbohydrate moiety (sialic acid) that extends into the aqueous phase. This arrangement further lends stability to the micelle. Casein micelles are stable under most heating, homogenization, and other dairy processing conditions.

Caseins possess a certain distinctive amino acid makeup, which impacts their processing and functional properties. They are rich in apolar and hydrophobic amino acids, namely valine, leucine, isoleucine, phenylalanine, tyrosine, and proline. The apolar amino acids are normally insoluble in water, but their nature is balanced by phosphate groups so that caseins exhibit some solubility. Methionine and cysteine, the sulfur-containing amino acids, are relatively low in caseins. This fact impacts their nutritional deficiency. On the other hand, the essential amino acid lysine content is high. In human diet, the high lysine content is helpful in complementing and balancing the low-lysine plant proteins. The ε-amino group of lysine present in caseins interacts with the aldehyde group of lactose at elevated temperatures, leading to the formation of brown pigments. This also explains browning of heat-sterilized milk and nonfat dry milk during extended storage.

The high prolene content results in low α-helix and β-sheet in their secondary structure, giving them ability to undergo more proteolytic degradation and enhanced digestion (Otter 2003).

Caseins possess limited secondary and tertiary structures. Accordingly, their molecular conformation is fairly flexible, and open. The polar and apolar amino acids in the primary structure of caseins contribute to hydrophilic and hydrophobic regions. This confers surface activity and contributes to the emulsifying and foam-forming characteristics of caseins.

Caseins are very heat stable under natural conditions of protein concentration, environmental pH, and ionic concentrations. Moderate heat has little or no effect on casein molecules, because they exist naturally in an open and extended state. However, heating of milk at an elevated temperature for an appreciable length of time could result in hydrolytic cleavage of peptide and phosphate bonds, which affects the stability of the complex, contributing to coagulation of milk.

Among the minor caseins of milk, γ-casein is the C-terminal fragment of β-casein, a product of attack by natural proteolytic enzyme plasmin. The N-terminal residue is the proteose–peptone fraction. These hydrolysis products of β-casein occur in the range of 3–10% of the total casein content of milk. The stage of lactation and health status of the cow affect their concentration.

Functional Peptides Derived from Casein. Peptides derived from caseins are biologically active and display significant extra nutritional attributes for maintaining normally of physiological functions in human subjects. Table 43.5 lists bioactive peptides originating from caseins.

TABLE 43.5 Some Functional Properties of Bioactive Peptides Derived from Caseins.

Peptide	Origin	Function
Casomorphins	β-caseins	Opioid agonists and ACE inhibitory/hypotensive
Casoxins	κ-casein	Opoid agonists
Casokinins	α- and β-caseins	Antihypertensive
Casoplatelins	κ-casein	Antithrombotic
Casecidin	α- and β-caseins	Antimicrobial
Immunopeptides	α- and β-caseins	Immunostimulants
Phosphopeptides	α- and β-caseins	Mineral carriers
Glycomacropeptide	κ-casein	Suppress appetite, antiplatelet, anticancer, antihypertensive, prevent dental caries, gingivitis, antiviral, antibacterial, bifidogenic

Source: Adapted from Pihlanto-Leppala (2003), Saxelin and others (2003), Aimutis (2004).

Functional peptides are generated during digestive processes in the body and during the fermentation processes used in fermented dairy foods. These peptides are inactive in the native proteins, but assume activity after they are released from them. They contain 3–64 amino acids, display largely hydrophobic character, and are resistant to hydrolysis in the gastrointestinal tract. They can be absorbed in an intact form to exert various physiological effects locally in the gut or may have a systemic effect after entry into the circulatory system. Casomorphins derived from milk caseins are known to be opioid agonists, and casoxins act as opioid antagonists. The opioids have analgesic properties similar to opium. Casokinins are antihypertensive (lower blood pressure), casoplatelins are antithrombotic (reduce blood clotting), and phosphopeptides are mineral carriers.

Casein phosphopeptides may aid in the bioavailability of calcium, phosphorus, and magnesium for optimum bone health. They may be helpful in preventing dental caries. They may also have a role in secretion of enterohormones and immune enhancement. The role of casein peptides in regulation of blood pressure is showing promise. Conversion of angiotensin I to angiotensin II is inhibited by certain hydrolyzates of casein and whey proteins. As angiotensin II raises blood pressure by constricting blood vessels, its inhibition causes lowering of blood pressure. This ACE inhibitory activity would therefore make dairy foods a natural functional food for controlling hypertension. A commercial ingredient derived by the hydrolysis of milk protein has an anxiolytic bioactive peptide with antistress effects. Psychometric tests and measurement of specific hormonal markers have displayed this antistress effect. The ingredient may be incorporated in milk, cheese, or ice-cream.

The glycomacropeptide released from κ-casein as a result of proteolysis may be involved in regulating digestion as well as in modulating platelet function and thrombosis in a beneficial way. It is reported to suppress appetite by stimulating CCK hormone. Consequently, it may be a significant ingredient of satiety diets designed for weight reduction. Furthermore, this peptide may inhibit binding of toxins in the gastrointestinal tract.

Some miscellaneous bioactive factors are being discovered. Specific proteins for binding vitamin B_{12}, folic acid, and riboflavin may assist in enhancing bioavailablity from milk and other foods. The fat globule membrane protein called butyrophilin is a part of the immune system. Other growth factors in milk may help gut repair after radiation or chemotherapy.

43.4.1.2 Whey/Serum Proteins. Whey proteins provide an excellent balance of essential amino acids. The amino acid profile resembles that of skeletal muscle, making the whey proteins effective in stimulating protein synthesis in adult muscle. Thus, they preserve muscle mass and enhance health. Whey proteins enhance fat loss. Whey proteins contain more branched-chain amino acids than any other protein, which are metabolized (to generate energy) in the muscle rather than in the liver. This property makes them suitable for use by athletes engaged in endurance sports like marathon racing. In general, it has been shown that whey proteins enhance humoral immune response. The sulfhydryl-containing amino acids, cysteine and glutathione, are related to the immune response. The branched-chain amino acids stimulate muscle glutamine synthesis. Glutamine is involved in immune function. Glutathione formation is facilitated by the high cysteine content of whey proteins, which in turn controls significant antioxidant defenses and immune function in the body.

Whey proteins are especially rich in cysteine. β-Lactoglobulin contains 33 mg of cysteine per gram of protein, and α-lactalbumin and bovine serum albumin contain 68 and 69 mg cysteine per gram of protein, respectively. The –SH compounds are also involved in quenching toxic free radicals.

In sports nutrition, the high content of arginine and lysine amino acids of whey proteins may help in stimulating the release of growth hormone, leading to an increase in muscle mass and decline in body fat. Furthermore, glutamine protects the immune system from decline caused by overtraining.

Whey proteins consist of β-lactoglobulin and α-lactalbumin, bovine serum albumin, immunoglobulins (mainly IgG1, IgG2, and IgM), lactoferrin, proteose–peptone, and a number of diverse enzymes.

β-Lactoglobulin. This major whey protein of milk displays the presence of four genetic variants. Besides, the two genetic variants A and B, variants C and D have also been reported. β-Lactoglobulin is rich in sulfur amino acids, containing five cysteine residues. It exists as a dimer linked by 1–3 disulfide bonds. It is a fairly heat-labile protein. β-Lactoglobulin stimulates lipolysis and so generates rancidity. It also acts as a carrier of vitamin A. The large numbers of lysine residues can result in lactosylation and accompanying changes in physical properties of the protein.

α-Lactalbumin. In human milk, α-Lactalbumin is a major protein, but in cow milk it is second in preponderance to β-lactoglobulin. Three genetic variants are reported, but Western cow contains variant B only. This protein is rich in tryptophan and the sulfur amino acids cysteine and methionine. There are four disulfides in the molecule and it exists as a monomer. α-Lactalbumin has 54 amino acid linkages, identical to the enzyme lysozyme. It is a glycoprotein as well as a metalloprotein. One molecule of calcium is bound to each protein molecule, which confers heat stability on α-lactalbumin. This protein has been shown to possess a physiological role in the synthesis of lactose in the mammary gland. It is a component of lactose synthetase along with uridine diphosphate-galactosyl transferase, catalyzing the transfer of galactose to glucose to form lactose.

α-Lactalbumin is a calcium-binding protein, therefore enhancing calcium absorption. It is an excellent source of essential amino acids such as tryptophan and cysteine.

Tryptophan regulates appetite, sleep–waking rhythm, and pain perception. Cysteine is important in functions of –SH compounds.

Immunoglobulins. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM. Their concentration is very high (100 g/L) in the first 2–3 milkings after calf birth, but falls to 0.6–1 g/L soon after. Immunoglobulins are antibodies synthesized in response to stimulation by specific antigens. These offer nonspecific humoral response to Gram-negative enteric and aerobic bacteria. Accordingly, they provide passive immune protection to the newly born calf. The basic structure of all immunoglobulins is similar, being composed of two identical light chains (23,000 daltons) and two identical heavy chains (53,000 daltons). The four chains are joined together by disulfide bonds. The complete molecule has a molecular weight of about 180,000 daltons. The antigenic sites are located at the $-NH_2$ terminal of the respective chain. Of the five immunoglobulin classes, IgG is the predominant fraction of milk, comprising about 90% of the total colostral immunoglobulins. Relatively smaller concentrations of IgM and IgA are also present in progressively decreasing amounts.

The immunoglobulins of milk are important for imparting an immune defense for the host. IgG1 is a major component. Other fractions are IgG2, IgA, and IgM, all of which provide passive immunity.

A number of colostrum products are being marketed for use as functional ingredients in foods. Colostrum contains several functional constituents including antibodies, lactoferrin, lactoperoxidase, cytokines, and growth factors. The antibodies act as antimicrobial agents against infection from rotavirus (which causes diarrhea), *Escherichia coli* (which causes food poisoning), *Candida albicans* (which causes yeast infection), *Streptococcus mutans* (which causes dental caries), *Clostridium difficile* (which causes antibiotic-associated diarrhea), *Cryptosporium parvum* (which causes food poisoning), and *Helicobacter pylori* (which causes ulcers and gastritis). Colostrum stimulates an active immune system by enhancing the activity of natural killer cells and phagocytes. The colostrum powder is manufactured by a special drying process to insure activity. Milk protein concentrate prepared from the milk of hyperimmunized cows is claimed to relieve joint pains from arthritis by complementing the body's naturally occurring anti-inflammatory substances.

Bovine Serum Albumin. As the name indicates, this protein originates from blood and, during synthesis in the udder, spills into milk. It is a large molecule with binding ability for fatty acids and metals.

Lactoferrin/Lactotransferrin. This is a glycoprotein, which displays strong tendency to bind ionic iron due to the presence of two metal-binding sites. An average lactoferrin content of 0.32 mg/mL has been found in cow milk. The molecular weight of lactoferrin varies between 73,700 and 74,000 daltons. Lactoferrin displays a very strong chelating tendency for ionic iron and forms a salmon-red-colored pigment. Lactoferrin is a single peptide chain, with two lobes, each of which is capable of binding iron. The iron-free form of lactoferrin is known as apolactotransferrin, which is colorless in appearance. Lactoferrin displays activity against several Gram-positive and Gram-negative bacteria, yeasts, fungi, and viruses. It particularly shows a strong inhibitory effect towards Gram--negative enteropathogenic bacteria by virtue of its ability to bind free ionic iron, which is essentially required for the growth of enteropathogenic microorganisms. In this way,

TABLE 43.6 Some Physiological Attributes of Peptides Derived from Whey Proteins.

Peptide	Origin	Function
β-Lactorphin α-Lactorphin	β-Lactoglobulin, α-Lactalbumin	Opioid agonist, inhibition of angiotensin-I-converting enzyme
Lactkinins	β-Lactoglobulin, α-Lactalbumin	ACE inhibitory (antihypertensive)
Lactoferricin	Lactoferrin	Antimicrobial activity
Lactoferroxins	Lactoferrin	Opioid antagonist

Source: Adapted from Harper (2000), Pihlanto-Leppala (2003), Aimutis (2004).

lactoferrin has a role in nonspecific defense of the host against invading pathogens. Apart from the antibacterial effect in the gut of the calf, a nutritional role in iron metabolism has also been ascribed to lactoferrin. Its iron-binding characteristic aids in enhancing iron absorption. It stimulates and protects cells involved in host defense mechanisms. Furthermore, it controls cytokine response.

Biologically Active Peptides from Whey Proteins. A number of peptides derived from whey proteins have physiological activity (Table 43.6). The bioactive peptides of whey proteins have been shown to exert a positive influence on body composition, satiety, and weight management. In addition, the bioactive peptides have ACE-inhibitory activity.

Milk Enzymes. Milk is a repository of a variety of enzymes. Over 60 indigenous enzymes have been reported in cow milk. They are either associated with the milk fat globule membrane (xanthine oxidase, sulfhydryl oxidase, and γ-glutamyltransferase), or with skim milk serum (catalase, superoxide dismutase), or with micelles of casein (plasmin and lipoprotein lipase).

Lactoperoxidase is an enzyme that breaks down hydrogen peroxide and exerts an antibacterial effect. Therefore, it is considered a natural preservative. Lysozyme has antimicrobial activity against Gram-positive bacteria and acts by lysis of cell walls. The Bifidobacteria flora of the colon imparts health-promoting properties and healthy gut ecology to the host. It has also been suggested that lysozyme may have an indirect effect on the defense systems as an immunomodulator through the stimulation of breakdown products of the peptidoglucan on the immunosystem.

43.4.2 Milk Fat

Milk fat in freshly secreted milk occurs as a microscopic globular emulsion of liquid fat in the aqueous phase of milk plasma. The composition of milk fat is given in Table 43.7. The milk fat of cows consists chiefly of triglycerides of fatty acids, which make up 95–96%. The remaining milk fat is composed of diglycerides, monoglycerides, free fatty acids, phospholipids, and cholesterol.

43.4.2.1 Physiological Effects of Milk Fat Components. The functional properties of milk fat are attributed to its fatty acid make-up. More than 400 distinct fatty acids have been detected in milk. Typical milk fat consists of 62% saturated, 29% monounsaturated, and 4% polyunsaturated fatty acids. It contains 7–8% short-chain fatty acids

TABLE 43.7 Constituents of Bovine Milk Lipids.

Lipid Fraction	Content in Milk (g/L)	Weight (%)
Triacylglycerols/triglycerides	30.7	95.80
Diacylglycerols/diglycerides	0.72	2.30
Monoacylglycerols/ monoglycerides	0.03	0.08
Free fatty acids	0.09	0.28
Phospholipids	0.36	1.11
Cholesterol	0.15	0.46
Cholesterol esters	0.006	0.02
Total	32.056	100.05

(C_4–C_8), which is a unique characteristic of milk fat (Table 43.8). Several positive findings have emerged for the consumption of milk fat.

Milk fat exists in an emulsion form in milk, making it highly digestible. Also, milk fat contains 10% short- and medium-chain fatty acids. Their 1 : 3 positions in the glyceride molecule allow gastric lipase with specificity for these positions to predigest them in the stomach itself. Butyric acid, a characteristic fatty acid of milk fat, is absorbed in the stomach and small intestine and provides energy similar to carbohydrates. Medium-chain fatty acids are transported to the liver for a rapid source of energy. The fatty acids lower the pH for facilitating protein digestion. At the same time, the acid barrier for pathogenic activity is enhanced. Free fatty acids and monoglycerides are surface-tension-lowering agents, thereby exerting an anti-infective effect.

Milk fat is a concentrated form of energy. Fat protects organs and insulates the body from environmental temperature effects. Milk fat functions as a source of fat-soluble vitamins A, D, E, and K and essential fatty acids, linoleic, and arachidonic acids. The essential fatty acids are not synthesized by the human body. They must be supplied by the diet. Arachidonic acid, with four double bonds, is present in traces. Its precursor is linoleic

TABLE 43.8 Milk Fat Fatty Acid Profile.

Fatty Acids	Common Name	Weight (%)
$C_{4:0}$	Butryic	3.8
$C_{6:0}$	Caproic	2.4
$C_{8:0}$	Caprylic	1.4
$C_{10:0}$	Capric	3.5
$C_{12:0}$	Lauric	4.6
$C_{14:0}$	Myristic	12.8
$C_{14:1}$	Myristoleic	1.6
$C_{15:0}$	–	1.1
$C_{16:0\ (branched)}$	–	0.30
$C_{16:0}$	Palmitic	43.7
$C_{16:1}$	Palmitoleic	2.6
$C_{17:0}$	–	0.34
$C_{18:0\ (branched)}$	–	0.35
$C_{18:0}$	Stearic	11.3
$C_{18:1}$	Oleic	27.42
$C_{18:2}$	Linoleic	1.5
$C_{18:3}$	Linolenic	0.59

TABLE 43.9 A Summary of Health Effects of Some Milk Fat Constituents.

Constituent	Physiological Effect
Butyric acid	Reduces colon cancer risk
CLA (conjugated linoleic acid)	Modulates immune function; reduces risk of cancer stomach, colon, breast, and prostate
Sphingolipids	May reduce risk of colon cancer
Stearic acid	May modulate blood lipids to reduce risk of cardiovascular and heart disease
Triglycerides	May enhance long-chain fatty acid and calcium absorption

Source: Adapted from Hoolihan (2004).

acid. Omega-3-linoleic acid and its products EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are also present in trace, but significant amounts. The positional location of individual fatty acids in the triglycerides is not random. In fact, the *syn*-1 and *syn*-2 positions on the glycerol molecule are mainly occupied by myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), or oleic acids ($C_{18:1}$). The *syn*-3 positions contain butanoic ($C_{4:0}$), hexanoic ($C_{6:0}$), or oleic ($C_{18:1}$) acids. A summary of major beneficial effects of milk fat is shown in Table 43.9.

Conjugated Linoleic Acids (*CLA*). Conjugated linoleic acids are a class of fatty acids found in animal products such as milk and yogurt. Rumen flora synthesizes CLA, which has been demonstrated to exhibit potent physiological properties. CLA is a strong antioxidant constituent of milk fat and may prevent colon cancer and breast cancer. It has been shown to enhance immune response. Prostaglandin PGE-2 promotes inflammation, artery constriction, and blood clotting. CLA may reduce the risk of heart disease by reducing levels of prostaglandin PGE-2. Studies have indicated that CLA may increase bone density, reduce chronic inflammation, and normalize blood glucose levels by increasing insulin sensitivity.

Phospholipids. A number of factors influence the unique phospholipids content of milk. The total phospholipids content of cow milk is approximately 2–5 mg/100 mL. They are located mostly in the fat globule and the membrane surrounding the fat in the globule.

Phospholipids are important because they are constituents of membranes and have a role in cell interaction with antibodies, ions, and hormones. They display surface-active properties and act as emulsifiers. Accordingly, they may improve fat absorption in the gastrointestinal tract. They are considered to protect gastric mucosa and may even extend protection from pathogenic microorganisms. Major components of phospholipids are 35% phosphatidyl ethanolamine, 30% phosphatidyl choline, 24% sphingomyelin, 5% phosphatidyl inositol, and 2% phosphatidyl serine. Sphingolipids are hydrolyzed in the gastrointestinal tract to ceramides and sphingoid bases, which help in cell regulation and function. Studies on experimental animals show that sphingolipids inhibit colon cancer, reduce serum cholesterol, as well as elevating the good cholesterol HDL. They could protect against bacterial toxins as well as infection.

Butyric acid is liberated from milk fat by lipase in the stomach and small intestine. It may exert a beneficial effect on the gastric and intestinal mucosa cells. In the colon, butyric acid is formed by fermentation of carbohydrates by the resident microbiota. Butyric acid in that location works as a substrate for colon cells and confers anticancer properties.

Cholesterol. Dietary total fat, saturated fat, and cholesterol are postulated to increase serum cholesterol, which is a risk factor for coronary heart disease. However, not all fatty acids cause elevation in total serum cholesterol. Stearic acid has a neutral effect, and lauric, myristic, and palmitic acids increase blood cholesterol levels. Individuals vary greatly in their blood lipids response to dietary fat and saturated fat. Also, it should be recognized that genetic factors are also responsible for synthesis of cholesterol in the liver; the bulk of serum cholesterol is infact controlled by genetic causes.

In general, the typical cholesterol content of whole milk (3.25% fat) is 10.4 mg/100 mL. It corresponds to 3–4 mg/g fat. In view of consumer preference for low-fat and nonfat dairy products, fat reduction is accompanied by cholesterol reduction. By separating fat from milk, an 80% reduction in cholesterol content can be achieved in skim milk. Thus, nonfat milk/skim milk show a residual cholesterol level of 4.9 mg/8 oz. serving.

43.4.3 Lactose

The content of the major carbohydrate of milk, lactose monohydrate, ranges from 4.8 to 5.2%. Lactose stimulates the absorption of calcium and magnesium. It has a relatively lower glycemic index of 46 as compared to 100 for glucose and 60 for sucrose (Gerdes 2002). This makes lactose in skim milk suitable for diabetics and in weight-control diets. It is less cariogenic than other sugars. Lactose stimulates bifidobacteria in the colon and thereby prevents infection and improves intestinal health.

Lactose absorption in humans is catalyzed by the enzyme lactase or β-D-galactosidase. Lactase is a nonpersistent enzyme in certain individuals, resulting in distressing symptoms of bloating, flatulence, and diarrhea following milk intake. Most individuals can tolerate two cups of milk spread over a day or with meals. In the case of lactose malabsorption, the symptoms are ameliorated by using lactase tablets or by consuming yogurt. Yogurt and some fermented milks contain live and active cultures, which on consumption by an individual furnish the enzyme lactase to assist in digesting lactose. Lactose-reduced milk and ice-cream products are also available.

A compound formed from lactose in heated milk products is lactulose. Heated milk contains up to 0.2% lactulose. As lactulose is not a digestible ingredient, it acts somewhat like a soluble fiber. Lactulose is generally used for treatment of constipation and chronic encephalopathy. Some recent data indicate that lactulose may enhance calcium absorption in the intestine. It stimulates the growth of *Bifidobacterium bifidum* and is thus beneficial in establishing useful microflora in the gut.

43.4.4 Minerals

Milk is an excellent source of minerals. The mineral content of milk is given in Table 43.10.

43.4.4.1 Functional Role of Calcium and Other Minerals. Milk and dairy products are excellent sources of bioavailable calcium. As compared with unfermented yogurt, addition of lactic acid to unfermented yogurt and its fermentation to regular yogurt, an improved bone mineralization has been observed. It is postulated that the acidic pH due to added lactic acid or naturally contained in fermented yogurt converts colloidal calcium to its ionic form and allows its transport to the mucosal cells of the intestine

TABLE 43.10 Major and Minor Minerals of Cows' Milk.

	Mean	Range
Major Mineral (mg/100 mL)		
Calcium, total	121	114–130
Calcium, ionic	8	6–16
Citrate	181	171–198
Chloride	100	90–110
Magnesium	12	9–14
Phosphorus, inorganic	65	53–72
Potassium	144	116–176
Sodium	58	35–90
Trace Elements (μg/100 g of milk)		
Boron	27	–
Chromium	1	0.8–1.3
Cobalt	0.1	0.05–0.13
Copper	20	10–60
Fluoride	12	3–22
Iodine	26	–
Iron	45	30–60
Manganese	3	2–5
Molybdenum	7	2–12
Nickel	2.5	0–5
Selenium	12	5–67
Silicon	260	75–700
Zinc	390	200–600

Source: Adapted from Swaisgood (1996), Fox (2003).

(Fernandes and others 1992). Milk supplies assimilable calcium and phosphorus in an optimum ratio. The major source of dietary calcium is dairy products, supplying as much as 75% of the dietary intake in developed nations. The bioavailability of calcium is further enhanced by the presence of vitamin D, lactose, and phosphoprotein (casein). One of the primary functions of calcium is to provide strength and structural properties to bone and teeth. Lack of adequate calcium intake, particularly during the growth phase, leads to osteoporosis or brittle bones in later life. It is also important in teeth development.

Calcium is involved in muscle contraction (including heart beat), blood coagulation, enzyme reactions, stimulation of hormonal secretions, and cell signaling. It is important in blood pressure control and is a factor in the prevention of colon cancer. Phosphorus is also critical in bone mass formation and takes part in various metabolic processes in the body. It is a crucial component of the genetic material DNA and RNA.

Iron is essential in the formation of hemoglobulin and in cytochrome activity. A deficiency causes anemia. Iron is further involved in brain function, in immunocompetence, and in the synthesis of lipids.

Magnesium is also a part of bone mass. It is involved in many metabolic pathways. Zinc is a component of several metabolic enzymes and DNA. It is involved in immune system functioning. Iodine is necessary for the formation of thyroid hormone, which regulates growth and metabolism. Copper is important in energy metabolism, as an antioxidant, is involved in collagen synthesis and iron utilization. Manganese is a cofactor of many metabolic enzymes. Chloride is an oxidizing agent and constitutes a vital ingredient of

stomach acid. Potassium is a major electrolyte in blood and tissues and helps in blood pressure regulation in conjunction with sodium. Sodium is further involved in nerve conduction, active transport, and bone formation.

43.4.5 Vitamins and Some Other Minor Constituents

In order to promote health and well-being, a balance of minerals and vitamins is required. They have to be supplied by food and supplements because they are not manufactured by the body. Milk contains both fat-soluble and water-soluble vitamins. The concentration of fat-soluble vitamins A, D, E, and K, and water-soluble vitamins B and C, and minor constituents of milk are given in Table 43.11.

Natural vitamin A activity in milk is due to retinol and the pigment β-carotene. Their level as well as those of vitamin D and E varies in milk according to the season and feed profile. Vitamin D is important in bone health and vitamin E is an antioxidant. Vitamin K is present in milk, but its dietary nutritional role is minor.

Milk is an important source of dietary B vitamins. They are stable to the various heating and processing conditions to which milk is normally subjected. Vitamin B_1, thiamin, is a cofactor in carbohydrate metabolism. Vitamin B_2 is involved in the oxidation reactions of glucose, fatty acids amino acids, and purines. Niacin facilitates utilization of carbohydrates, fat synthesis, and tissue respiration. Pantothenic acid participates in fatty acid metabolism. Vitamin B_6 is critical in protein metabolism. Folic acid acts as a growth factor and is involved in DNA synthesis. Vitamin B_{12} is required for growth, blood formation, and nerve tissue functioning. Biotin has a role in metabolism of carbohydrates, lipids, nucleic acid, and proteins. Ascorbic acid (vitamin C) is necessary for collagen formation, healing of wounds, and absorption of nonheme iron. It provides resistance to infections. However, vitamin C content of milk is very low and insignificant.

43.4.6 Enhancement of Functional Properties by Culturing of Milk

Fermented milks like yogurt are enhanced functional foods due to the fact that they contain nutrients of milk as well as products of metabolic activities of starter microorganisms in

TABLE 43.11 Vitamins of Milk.

Vitamins	Concentration in 100 mL Milk
A	40 μg RE*
D	4 IU**
E	100 μg
K	5 μg
B_1	45 μg
B_2	175 μg
Niacin	90 μg
B_6	50 μg
Pantothenic acid	350 μg
Biotin	3.5 μg
Folic acid	5.5 μg
B_{12}	0.45 μg
C	2 mg

*Retinol equivalent.
**International units.

the product. Furthermore, they contain live and active cultures in significant numbers to effect physiological benefits to the consumer. Bacterial mass content and the products of the lactic fermentation further distinguish yogurt from milk.

43.4.6.1 Probiotics. Probiotics may be defined as a food or supplement containing concentrates of defined strains of living microorganisms that on ingestion in certain doses exert health benefits beyond inherent basic nutrition. Probiotics and associated ingredients might add an attractive dimension to cultured dairy foods for effecting special functional attributes.

Milk is an excellent medium to carry or generate live and active cultured dairy products (Chandan 1999; Shah 2001; Mattila-Sandholm and Saarela 2003). Fermentation adds an attractive dimension to cultured dairy products for augmenting the current demand for functional foods. The buffering action of the milk proteins keeps the probiotics active during their transit through the gastrointestinal tract. In general, worldwide consumption of fermented milk products has increased due to their high nutritional profile, unique flavor, desirable texture, and remarkable safety against food-borne illness.

43.4.6.2 Beneficial Microflora. Cultures associated with health benefits are yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*), other lactobacilli, and bifidobacteria (Chandan 1999). Table 43.12 gives a list of various probiotics being used in commercial fermented milks. Yogurt organisms possess a distinctly high lactase activity, making it easily digestible by individuals with

TABLE 43.12 Probiotic and Beneficial Microorganisms in Commercial Products.

Lactobacillus acidophilus
Lactobacillus johnsoni LA1
Lactobacillus gasseri ADH
Lactobacillus crispatus
Lactobacillus casei/paracasei
Lactobacillus casei subsp. *Rhamnosus*
Lactobacillus reuteri
Lactobacillus brevis
Lactobacillus delbrueckii subsp. *bulgaricus*
Lactobacillus fermentum
Lactobacillus helveticus
Lactobacillus plantarum
Bifidobacterium adolescentis
Bifidobacterium animalis
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium infantis
Bifidobacterium longum
Streptococcus thermophilus
Enterococcus faecium
Pediococcus acidilactici
Saccharomyces boulardii

Source: Adapted from Chandan (1999), Saxelin and others (2003), Shah (2004).

TABLE 43.13 Potential Health Benefits of Fermenting Milk with Probiotics.

Effects Corroborated by Scientific Evidence	Effects of Potential Nature
Assisting lactose digestion	Controlling Candida and bacterial infections (vaginitis)
Treatment of rotaviral diarrhea	Alleviating constipation
Treatment of infant gastroenteritis	Antimutagenic/anticarcinogenic effects
Treatment of antibiotic-related diarrhea	Lowering cholesterol and blood pressure
Modulating intestinal (microbiota) ecology	Alleviation of microbial overpopulation in small intestine
Reducing harmful fecal enzymes, biomarkers of cancer initiation	Lowering of cholesterol and controlling hypertension
Enhancing/modulating immune system	Prevention and treatment of Crohn's disease
Positive effects on cervical and bladder cancer	Treatment of *Clostridium difficile* diarrhea
Reducing symptoms of atopic dermatitis in children	Prevention of sexually transmitted disease like AIDS

Source: Adapted from: Chandan (1999), Shah (2001), Fonden and others (2003).

a lactose-maldigestion condition. To bolster probiotic function, most commercial yogurt is now supplemented with *Lactobacillus acidophilus* and *Bifidobacterium* spp.

The probiotic preparations are also available in the form of tablets, powder, or capsules. They contain organisms from the genera *Lactobacillus*, *Enterobacter*, *Streptococcus*, and *Bifidobacterium*. These genera are important members of the gastrointestinal microflora and are all relatively beneficial. The strains of lactic acid bacteria used in probiotics are mostly intestinal isolates such as *L. acidophilus*, *L. casei*, *E. faecium*, and *B. bifidum*.

Yogurt starter bacteria, *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*, are also included as probiotics in this table because yogurt has been associated with several health benefits in the past. They are now reported to persist and remain viable throughout the human gastrointestinal tract. The continuous ingestion of live products ensures abundant numbers to maintain their functional status. Even with intestinal isolates such as *L. acidophilus*, it is necessary to dose regularly rather than to assume that a few doses will allow the organisms to colonize the gut permanently. Currently, known probiotics are fortified with enzymes, anti-inflammatory compounds, specific amino acids, colostrum, and chelated minerals in probiotic preparations. *L. acidophilus* and *B. bifidum* strains are known to differ widely in their ability to grow in the presence of bile salts. Both are reported to be stable at various concentrations of bile salts.

Potential health benefits of probiotics are given in Table 43.13. Accordingly, as a result of fermentation, the health benefits of milk are considerably further enhanced.

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44

Functional Foods Based on Meat Products

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44.1 Introduction 990
44.2 Meat Components and Health 991
44.2.1 Proteins 991
44.2.2 Lipids 991
44.2.3 Micronutrients 993
44.2.4 Other Components 994
44.3 Meat and Meat Products as Functional Foods: Technologies and Strategies 994
44.3.1 Improvement of Meat Components for Designing Meat-Based Functional Foods 994
44.3.1.1 Animal Production Practices for Meat-Based Functional Food Production 995
44.3.1.2 Meat Transformation Systems Tailored to Meat-Based Functional Food Production 999
44.3.1.3 Distribution and Storage Conditions 1008
44.3.1.4 Influence of Preparation and Consumption 1008
44.3.2 Bioavailability Considerations 1008
44.3.2.1 Synergistic Effect of Absorption of Nutrients in Muscle Foods 1009
44.3.2.2 Effect of Processing on Bioavailability of Meat Product Components 1009
44.4 Conclusions 1009
References 1010

[Note: All information in this chapter is the most current scientific and technical assessment and is unrelated to the legal applications, if any, in individual countries.]

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44.1 INTRODUCTION

Meat and meat products are valuable components of the human diet and the meat industry is one of the most important industries in the world economy. Annual meat production is projected to increase from 218 million metric tons in 1997–1999 to 376 million metric tons by 2030 (WHO 2003). In the past, meat has been considered an important food with great nutritional value. Consumption of meat was associated with good health and prosperity and the consumer selected meat products mainly based on sensory factors. Today, changes in dietary and life-style patterns have refocused consumer criteria to choose meats that will promote health and quality of life. This fact has enormous implications owing to the intense competition in the food industry, which makes it extremely sensitive to consumer demands and perceptions.

Nutrition is now recognized as a major modifiable determinant of noncommunicable chronic diseases. There is increasing scientific evidence supporting the view that diet alterations have strong positive and negative effects on health throughout life (WHO 2003). It is in this context that the so-called *functional foods* have emerged and have come to represent one of the fastest growing segments of the world food industry.

Presently there is no universally accepted definition; however, a working definition has been established in the consensus document concerning Scientific Concepts of Functional Foods in Europe. Functional food is more a concept than a well-defined group of food products that fits the following definition: "A food can be regarded as a functional food if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to an improved state of health and well-being and/or reduction of risk of disease. Functional foods must remain foods and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet: they are not pills or capsules, but part of a normal food pattern" (Diplock and others 1999).

The meat industry has been slow to follow the functional trends, despite the fact that it is one of the sectors whose development may be of major interest. Over the last several decades, one of the reasons meat products have come under increasing scrutiny by medical, nutritional, and consumer groups is because of the associations between the consumption of meat constituents (i.e., fat, cholesterol) and the risk of major society diseases (i.e., ischemic heart disease, cancer, hypertension, and obesity). Based on nutrient and dietary goal recommendations, meat-based functional foods have the opportunity to improve their image and better serve the needs of consumers. Aside from meat's current relative importance, meat also constitutes a means for achieving a needed diversification in taking a position in emerging markets with great implications for the future. Therefore, developments in food processing and technology that enable the production of functional foods are increasingly important.

The beneficial effects of functional foods are associated with the role of one or more physiologically active components (functional or bioactive components). Knowledge of these substances is essential for the identification, design, and development of functional foods in general, and meat-based functional foods in particular. This chapter will address the functional foods based on the different strategies that the meat industry can utilize to affect potential functional components. The production of functional meat products requires the fullest possible understanding of the health implications, both positive and negative, of meat components. It is only through this

knowledge that it is possible to devise proper strategies for the modulation of their characteristics as needed.

44.2 MEAT COMPONENTS AND HEALTH

Foods are made up of thousands of biologically active components that have the potential to cause functional effects (improvement of health status and well-being and/or reduction of risk of disease). Although most of the biologically active components are produced by plants, some are also found in meat and meat products. These components include important sources of highly available forms of proteins, vitamins, and minerals. Meat contains bioactive constituents known to have protective effects (Arihara 2004; Biesalski 2004). Meats also contribute to components that, when ingested in excess, can have unhealthy implications (e.g., fat, saturated fatty acids, cholesterol, salt).

44.2.1 Proteins

Meat is a fundamental source of proteins of high biological value. Meat provides a well-balanced source of amino acids that satisfies human physiological requirements such as tissue growth and reconstruction. Supplying sufficient amino acids to maintain the protein reserves of the body is also an important factor in antibody synthesis, thus promoting acquired immunity to disease (Romans and others 1994). Certain amino acids present in meat have favorable effects on the nervous and immune systems. Recently it has been identified that some peptides released either during food processing or during digestion are related to reducing the risk of cardiovascular disease (CVD) or hypertension and alleviating the effects of alcohol (Garnier 2004).

44.2.2 Lipids

A variety of evidence has led to the establishment of a relationship between dietary fat and obesity, CVD, and certain types of cancer (WHO 2003). To prevent diet-related chronic diseases, the following goals for dietary nutrient intake have been established: Total dietary fat should provide between 15 and 30% of the calories consumed, with saturated fatty acids (SFA) supplying less than 10% of said calories, polyunsaturated fatty acids (PUFA) from 6% to 10% (ω-6, 5–8%; ω-3, 1–2%), and trans fatty acids less than 1%, while cholesterol intake should be limited to less than 300 mg/day (WHO 2003). Meat and meat products are important sources of dietary fat. The fat content in meat can vary widely depending on factors such as species, type of cut, and extent of fat trimming (carcass processing, primal cuts, retail cuts), among others. Meat lipids usually contain less than 50% SFA (of which only 25–35% have atherogenic properties) and up to 65–70% unsaturated (monounsaturated, MUFA, and PUFA) (Table 44.1). Ruminant meat is also a source of trans fatty acids, which are formed during biohydrogenation in the rumen.

Conjugated linoleic acid (CLA), a mixture of geometric and positional isomers of linoleic acid, is only found in useful amounts in meat and milk, especially from ruminants. The majority of the CLA present is in the form of 9-*cis*, 11-*trans* octadecadienoic acid. Its importance as a functional component lies in the fact that it appears to behave as an anticarcinogenic and antiatherogenic agent, and it induces a decrease in body fat and an increase in protein content. Products obtained from ruminants constitute the main

TABLE 44.1 Composition of Selected Meats and Meat Products[a].

Product	Fat (g)	SFA[b] (g)	MUFA (g)	PUFA (g)	Calories (kcal)	Cholesterol [C] (mg)	Sodium (mg)	Fe (mg)	Se (μg)
Pork (loin)	5.66	1.95 (38.0)	2.56	0.61	143 (35.6)	59	52	0.84	36.1
Beef (top round)	3.37	1.15 (42.4)	1.42	0.14	129 (23.5)	46	64	1.97	31.9
Lamb (leg)	4.19	1.50 (42.0)	1.69	0.38	125 (30.1)	64	61	1.82	23.4
Chicken (breast)	1.24	0.33 (36.2)	0.30	0.28	110 (10.1)	58	65	0.72	17.8
Frankfurter	27.64	10.77 (39.6)	13.67	2.73	305 (81.5)	50	1120	1.15	13.8
Frankfurter (low fat)	10.00	3.69 (39.6)	4.69	0.93	154 (58.4)	44	1257	1.20	15.1
Bologna	24.59	9.67 (45.3)	10.52	1.12	304 (72.8)	60	736	1.21	26.1
Pork sausage	26.53	8.79 (36.4)	11.81	3.55	304 (78.5)	72	636	1.11	0.0
Salami	34.39	12.20 (37.5)	17.10	3.21	418 (74.0)	79	1860	1.51	26.1
Bacon	45.04	14.99 (37.6)	20.05	4.82	458 (88.5)	68	833	0.48	20.2
Cooked ham	2.37	0.79 (28.5)	1.67	0.31	122 (17.4)	22	900	0.39	10.4

Source: USDA (2004).
[a]Amount in 100 g of edible portion.
[b]In parentheses, % of total fatty acids.
[c]In parentheses, % of calories from fat.
SFA: saturated fatty acids; MUFA: monosaturated fatty acids; PUFA: polyunsaturated fatty acids.

source of CLA. Beef fat contains 3.1–8.5 mg/g fat (Hasler 1998) and lamb fat about 6 mg/g fat (Cassens 1999), while the lowest levels are found in the tissues of monogastric animals: 0.6 mg/g fat for pork (Higgs 2000) and chicken (Takenoyama and others 1999).

The amount of cholesterol in meat and meat products depends on numerous factors. In general, there is less than 75 mg cholesterol/100 g meat or meat product (Table 44.1). The exceptions are some edible offal (heart, kidney, brain, and so on) where the concentrations are much higher (Chizzolini and others 1999).

44.2.3 Micronutrients

Many of the apparent beneficial effects of animal source foods on human health and function are mediated in part by the micronutrients they contain. Meat is a good source of iron, zinc, and phosphorus, with significant amounts of other essential trace elements such as selenium, magnesium, and cobalt. Meat constitutes an excellent, highly bioavailable source of iron (Table 44.1); 50–60% of this iron comes in the heme form and contributes about 14–22% of total dietary iron intake (Schweitzer 1995; Higgs 2000). Iron deficiency is one of the most prevalent nutritional deficiencies worldwide, both in developing and developed nations (Neumann and others 2002). Menstruating women in particular constitute a group at risk for iron deficiency. Surveys carried out in France and North America reported iron deficiency in nearly 20% of these women (O'Sullivan and others 2002). A reduction in the consumption of meat of 50% could result in an excessively low iron intake (less than 8 mg/day) in one-third of women. This fact raises serious questions about the appropriateness of the general message to reduce meat intake, especially in certain populations (Carbajal 2004).

Meat is the richest food source of zinc, supplying approximately 20–40% of the amount absorbed (Higgs 2000). Increasing attention is being paid to the global prevalence of mild to moderate zinc deficiency both in developing countries and in disadvantaged groups in industrialized countries. Because of the widespread effects of zinc deficiency on morbidity, mortality, growth, and development, policy-makers must pay much more attention to improving diet quality through food-based approaches or supplementation where needed to address severe deficiency (Neumann and others 2002).

Selenium is one of the major antioxidants considered to protect against coronary heart disease and cancers (Higgs 2000). Table 44.1 shows that meat also contains significant amounts of selenium and provides about 25% of the daily requirement of this essential mineral.

Meat and meat products are an excellent source of the B-group vitamins thiamine (B_1), riboflavin (B_2), niacin, pantothenic acid, vitamin B_6, and vitamin B_{12}. Meat does not contain significant amounts of vitamins A, C, D, E, or K, although vitamin A is abundant in certain organs (liver, kidney). Recent analyses of meat and liver reveal significant amounts of 25-hydroxycholecalciferol, assumed to have a biological activity five times that of cholecalciferol. This seems to indicate that the role of meat and meat products in vitamin D intake has been underestimated, and that the importance of meat in the prevention of rickets in children and osteomalacia in adults, as well as its effect on bone metabolism, should be reviewed (Higgs 2000). Meat is an important source of folate; a folate-deficient diet has been associated with increased risk for different types of cancer resulting from low intake of fruits and vegetables. However, it has been considered that the bioavailability of folate from meat and liver is much better than that from fruits and vegetables (Biesalski 2004).

Meat also contains other micronutrients such as carnitine, creatine, choline, and so on; their importance in human physiology is only just beginning to be recognized (Garnier 2004).

44.2.4 Other Components

Meat is one of the richest natural sources of glutathione, which is an important reducing agent providing a major cellular defense against a variety of toxicological and pathological processes. The importance of glutathione in the defense against chronic disease signals a positive potential for meat (Higgs 2000). Natural polyamines (putrescine, spermidine, and spermine), which are ubiquitous components of meat, are involved in a multitude of basic metabolic processes with significant implications for human health; for example, they are essential for the maintenance of the high cell turnover rate of organs such as the gastrointestinal tract, pancreas, and spleen or of the high metabolic activity of the normally functioning and healthy gut. However, in other cases, the intake of polyamines should be minimized in an attempt to slow down the growth and progress of a tumor (Bardócz 1995).

44.3 MEAT AND MEAT PRODUCTS AS FUNCTIONAL FOODS: TECHNOLOGIES AND STRATEGIES

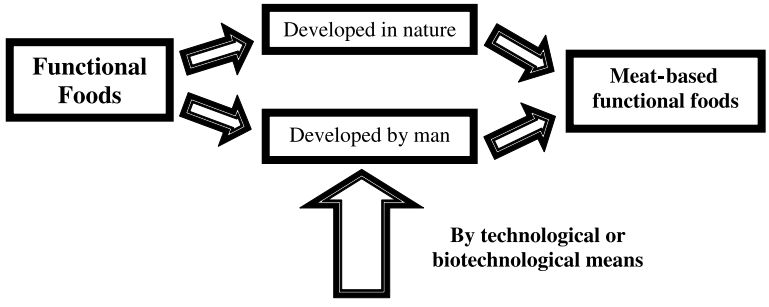
A functional food can be a natural food, a food to which a component has been added, or a food from which a component has been removed by technological or biotechnological means. It can also be a food in which the nature of one or more components has been modified, one in which the bioavailability of one or more components has been modified, or any combination of these possibilities (Diplock and others 1999). Functional foods include whole foods and fortified, enriched or enhanced foods (Hasler and others 2004). A number of the approaches for functional food production (Roberfroid 2000; Jiménez-Colmenero and others 2006) can be applied to meat processing (Fig. 44.1).

There are a number of ways to alter the presence of different functional components that can result in the development of meat-based functional foods. Some strategies used to achieve this are: genetic and nutritional, implemented through animal production practices; strategies dependent on transformation systems (preparation of meat raw materials, reformulation and processing), relative to distribution and storage; and finally, targeting the conditions in which products are consumed (Jiménez-Colmenero and others 2006).

Described in the following is an impact analysis of these strategies on the functional components, as well as the procedures followed to improve meat and meat product composition, and procedures that affect the bioavailability of certain components with functional effects. Likewise, the formation of some components whose presence may cause a deterioration of health status and well-being and increase the risk of disease are considered.

44.3.1 Improvement of Meat Components for Designing Meat-Based Functional Foods

From the farm to the table, there are a number of stages in which, either intentionally or incidentally, the composition of meat and meat products can be changed . These changes affect the presence of several functional components (Fig. 44.2).



- ELIMINATING/REDUCING a component known to have deleterious effects.
- INCREASING the concentration of a component naturally present in food (nutritive or nonnutritive) to a level known to produce beneficial effects.
- ADDING a component (macro or micronutrient or nonnutrient) that is not normally present in food but the beneficial effects of which have been demonstrated.
- REPLACING a component, usually macronutrient, whose intake is usually excessive and thus a cause of a deleterious effect, by a component for which beneficial effects have been demonstrated.
- MODIFICATION of the nature or bioavailability of one or more components with beneficial effects.
- COMBINATION of these possibilities.

Figure 44.1 *Approaches for meat-based functional food production.*

44.3.1.1 Animal Production Practices for Meat-Based Functional Food Production. Animal production practices represent the first opportunity to condition the presence of functional components. The composition of animal tissues, and hence carcasses and commercial cuts (and meat raw materials), varies not only according to species, but also according to breed, age, sex, type of feed, and so on. Several strategies are available for inducing changes (*in vivo*) in meat constituents. These strategies include genetic selection, gene manipulation, nutrition and feeding management, growth-promoting and nutrient-partitioning agents, and immunization of animals against target circulating hormones or releasing factors.

Lipid Modification. As meats (and meat products) are among the major sources of dietary fat, the attempt to approximate meat lipid characteristics (quantitative and qualitative) to recommended dietary nutrient goals is considered essential.

FAT CONTENT REDUCTION. The fat content in meat has significantly decreased in recent decades. Carcass fat has been reduced by about 6–15% in beef, 15–30% in pork, and 10% in lamb. Further reductions are anticipated for beef and lamb over the next few years (Goutefongea and Dumont 1990; Higgs 2000). The extent of and method for accomplishing the reductions depend on the species.

Although animal fat has been reduced by traditional genetic means (selective breeding), new alternatives have emerged with some of the genetic manipulation techniques more recently made available. Genomic maps of farm animals used for meat production have lately been constructed. This will enable the determination of regions within the

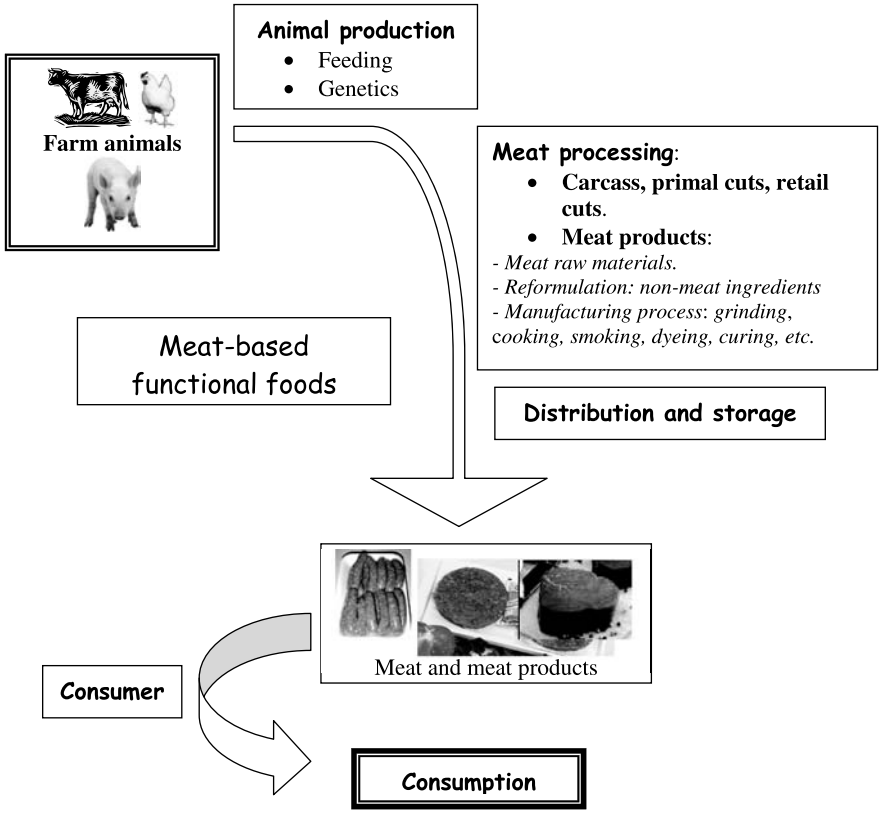


Figure 44.2 *From farm to table: strategies to affect meat and meat products components with potential implications in human health.*

genome that contain one or more genes with potential implications for quantitative parameters (fat distribution) of interest for selection (Kirton and others 1997; Barroeta and Cortinas 2004).

Carcass fatness can be manipulated in cattle, sheep, pig, and poultry by production practices. Nutritional strategies are easier to apply in monogastric animals than in ruminants. These strategies are determined largely by the composition of diets and by the levels of feeding, particularly the energy and protein intake (Hays and Preston 1994; Dikeman 1997). Using partitioning agents like anabolic steroids, growth hormones, and so on, or immunization strategies, it is possible to alter some metabolic processes that regulate the utilization of nutrients during growth in order to promote protein synthesis and reduce fat deposition (Bass and others 1990; Byers and others 1993). Additional management options to reduce carcass fat include elimination of castration, selection according to maturity, size, and sex, and others (Bass and others 1990; Dikeman 1997).

CHANGES IN FATTY ACID COMPOSITION. There are several ways to modify the fatty acid composition in farm animal fat, although the extent and procedure depend on the species. Genetic methods now enable the modification of fatty acid composition, specifically the

contents in palmitic, palmitoleic, myristic, linoleic, and linolenic acids, of pork (Clop and others 2003).

Lipid deposition in animal tissues can be endogenous, that is, synthesized *de novo*, or exogenous, caused by diet. Dietary fatty acid composition is an extremely important part of the fatty acid profiles of monogastric animals (pigs, poultry) and less important in ruminants (cattle). For this reason, numerous animal feeding trials have been carried out in the attempt to make the meat fatty acid composition more consistent with current human health recommendations and consumer requirements. Feeding strategies in beef, pork, lamb, or chicken have shown that plant (vegetable oils, plants rich in ω-3, forages) and marine sources (fish oil or fish meal) have been successfully used to significantly increase the levels of ω-3 PUFA (up to six-fold) and, more specifically, of eicosapentaenoic acid (EPA, 20:5ω3; e.g., up to 10-fold in beef), docosahexaenoic acid (DHA, 22:6ω-3; e.g., up to 60-fold in chicken), and linolenic acid (18:3ω-3) (Enser 2000; Sloan 2000; Barroeta and Cortinas 2004; Raes and others 2004). With these strategies, 100 g of chicken meat can provide 90% of the recommended daily allowance of EPA + DHA (Barroeta and Cortinas 2004).

Epidemiological, clinical, and biochemical studies have provided evidence of the chemopreventive activity of these fatty acids against some of the more common cancers (breast and colon cancer), rheumatoid arthritis, inflammatory bowel diseases, and CVD (Hoz and others 2004). Dietary modification of fatty acid composition has also made it possible to lower the ω-6/ω-3 ratios in farm animal tissues. This is important because evidence has been accumulated that suggests that increased intake of ω-6 and associated relative ω-3 deficiency, not cholesterol, is the major risk factor for cancers, coronary heart disease, and cerebrovascular disease (Okuyama and Ikemoto 1999).

Dietary supplementation has been used to enrich chicken, pork, beef, and lamb with CLA (Enser 2000; Lynch and Kerry 2000; Barroeta and Cortinas 2004; Raes and others 2004). Meat containing CLA has been described as a functional food (Pennington 2002; Hasler and other 2004).

REDUCTION OF CHOLESTEROL. Selecting animals with the right genetics, feeding them diets rich in unsaturated fats and treating them with growth-promoting or repartitioning agents are the most common strategies for modifying the cholesterol content in living animals. However, most of them have a negligible impact on cholesterol levels (Clarke 1997). Although variations can be observed among species, muscles, or certain breeds, between sexes or in relation to certain feeding regimes, their magnitude generally appears to be too low for them to be of real use in the diet-related reduction of cholesterol intake (Chizzolini and others 1999). The most promising method for selective reduction of the muscle tissue cholesterol content in a living animal (by 20.4% in breast muscle) appears to be through the provision of elevated dietary copper (Clarke 1997).

Vitamins. Increasing the presence of unsaturated fatty acids in meat causes heightened susceptibility to oxidation, a process that leads to undesirable changes in sensory characteristics. Food lipid oxidation is considered to pose a risk to human health. Some lipid oxidation products are considered atherogenic and appear to have mutagenic, carcinogenic, and cytotoxic effects (Chizzolini and others 1998). There are several means of minimizing lipid oxidation associated with animal feeding. These means represent the only technologies available to alter the oxidative stability of intact muscle foods.

Supplementing with dietary vitamin E (α-tocopherol) significantly in excess of physiological levels reduces lipid oxidation as well as myoglobin oxidation in the meats of poultry, pigs, cattle, and rabbits. The accumulation depends on species, muscle characteristics, levels of supplementation, and duration of feeding. (Morrissey and others 1998; Lynch and Kerry 2000; Dal Bosco and others 2001).

In addition to improving muscle quality, this strategy converts meat from a poor source of vitamin E to at least a moderate one (Lynch and Kerry 2000). It has been suggested that just 100 g of chicken can supply up to 30% of the recommended vitamin E intake (Barroeta and Cortinas 2004). Tocopherols are known for their efficient antioxidant activity in foods and biological systems; their ingestion helps to potentiate the antioxidative capacity of the organism (Surai and Sparks 2001). Epidemiological studies have provided evidence of an inverse relationship between coronary artery disease and vitamin E supplementation (Pryor 2003).

Dietary supplementation with the remaining fat-soluble vitamins or the water-soluble vitamins does not significantly change their concentration in muscle (Lynch and Kerry 2000).

Minerals. Apart from selenium and, to a lesser extent, iron, these muscle minerals are not responsive to dietary supplementation (Lynch and Kerry 2000).

Supplementation of the farm animal diet with iron increases muscle iron. However, diets low in iron produce anemic animals and the paler meat that is preferred by consumers (Lynch and Kerry 2000). It has been suggested that the use of supplemental iron (from hemoglobin) to increase pig meat iron levels could play an important role in reducing iron deficiency in some risk groups (O'Sullivan and others 2002; Ramírez and others 2002).

Dietary selenium supplementation makes it possible to increase the selenium levels in pork, beef, veal, and poultry (Wenk and others 2000; Surai and Sparks 2001). This strategy is presently utilized in most farm animal diets (Wenk and others 2000) and produces high-selenium pork that contains about ten times the selenium content of traditional pork (Anon. 2000).

The elimination of supplemental copper from the diet of broilers improves the oxidative stability of the meat, although the muscle copper concentration is not affected (Morrissey and others 1998).

Other Components. As animals cannot synthesize carotenoids, their presence in tissues depends completely on diet. They are absorbed from the food and transferred along the food chain. Carotenoids are used in animal feeds principally to enhance the color of poultry meat, eggs, and the flesh of some species of fish. Their potential role as antioxidants has increased the interest in achieving their incorporation into animal tissue (Morrissey and others 1998; Lynch and Kerry 2000). Carotenoids have been related to CVD risk reduction, cancer prevention, and immune function enhancement in mammals (Torrissen 2000). Diets enriched with different carotenoids (β-carotene, lycopene, zeaxanthin, lutein, and so on) have been employed to feed poultry (Morrissey and others 1998; Torrissen 2000; Sagarra and others 2001).

Inclusion in animal diets of other compounds with potential antioxidant activity (ubiquinone, plant phenolics, glutathione, phytoestrogens, carnosine, carnitine, and so on) has been described as a method to increase the oxidative stability of muscle food

(Decker and Xu 1998; Lynch and Kerry 2000). Feeding pigs certain types and amounts of soybean meal increases the amount of isoflavin in the meat, a circumstance that should justify the claims that pork is a functional meat as isoflavones have been shown to improve cardiovascular health in humans (Quaife 2002).

44.3.1.2 Meat Transformation Systems Tailored to Meat-Based Functional Food Production. Meat processing is another level at which it is possible to introduce changes in the amounts and types of functional components in processed meats (Table 44.2). Several basic approaches (Fig. 44.2) can be used to successfully induce the desired effects (Jiménez-Colmenero and others 2006). These measures focus on the treatment of the ingredients in order to secure a raw material suitable in terms of composition, reformulation of meat products to induce certain changes in their composition, and

TABLE 44.2 Approaches for Improving the Composition of Meat-Based Functional Products.

Reducing
• Lipid components: – Total fat – Saturated fatty acids – *Trans*-monosaturated fatty acid – *Trans*-polyunsaturated fatty acids – ω-6/ω-3 PUFA ratio – Cholesterol
• Sodium
• Unhealthy compounds (formed during processing, distribution, storage or consumption): nitrosamines, biogenic amines, polycyclic aromatic hydrocarbons, heterocyclic amines, lipid oxidation products
Replacing
• Lipid: animal fat by vegetable and fish oils
Increasing
• Lipid fraction – *cis*-monounsaturated fatty acids – ω-3 polyunsaturated fatty acids (linolenic acid 18:3ω-3; eicosapentaenoic acid, 20:5ω-3; docosahexaenoic acid, 22:6ω-3) – Conjugated linoleic acid
• Vitamin E
• Minerals (Ca, Se, Fe, Mg, Mn)
• Other compounds
Adding
• Plant-based protein
• Dietary fiber
• Probiotics
• Carotenoids
• Vitamin C
• Plant sterols
• Phytate
• Other substances

adaptation of the processing technologies. There are numerous aspects to be taken into account in the development of the new meat product and they all must respond to the same quality criteria (technological, sensory, nutritional, safety, convenience, and so on) as any other product.

Modification of the Meat Raw Material Composition. Whether for direct consumption or transformation into meat products, meat can be subjected to different treatments that modify the composition, generally regarding fat and cholesterol. The desirability of limiting the fat content in commercial cuts and meat products has encouraged the development of various procedures designed to separate and/or extract both visible fat and fat that is located in less accessible parts of the muscle tissue The most immediate system consists in extensive trimming to remove external and internal fat from the carcass; further trimming is done on primal cuts and, where necessary, the defatting can be completed on retail cuts. The final fat percent is limited by the intramuscular fat content and in some cases is truly low.

In products involving more structural breakdown, a number of procedures have been developed to reduce the percentage of fat in meat raw materials. These procedures require the reduction of meat particle size, followed by a preparatory phase (modification of pH, ionic strength of medium, and so on) prior to actual separation or removal using cryoconcentration, centrifugation, decantation, and so forth (Giese 1992; Jiménez-Colmenero 1996). Supercritical fluid extraction to remove fat and cholesterol from meat has been performed with beef, pork, and chicken. This innovative technique is very effective when the meat is partially dehydrated, with fat and cholesterol extraction even surpassing 90–95%. At normal moisture levels, the results of supercritical fluid extraction have been poor (Clarke 1997).

Modification of the Formulation Process. The most versatile manner of modifying the composition of meat products includes a wide range of options for changing the ingredients employed in their preparation and, consequently, these options alter the content of different endogenous and exogenous bioactive compounds. This strategy makes it possible to apply a number of the approaches proposed for the production of functional foods: reducing, increasing adding and/or replacing different functional components (exogenous and endogenous) (Fig. 44.1). The technological difficulties involved depend on the type of product (composed of identifiable pieces of meat, coarsely or finely ground, emulsions, heat treatment, curing, and so on) and on the modification to be introduced. The different types of reformulation approaches for designing meat-based functional foods, many of which can be applied simultaneously, are analyzed below.

REDUCTION OF COMPONENTS. Because some components normally present in meat and meat products have been associated with the development of certain diseases, the first approach to the reduction of disease risk (functional effect) involves reducing their concentration to appropriate limits (Table 44.2).

Reduction of Fat and Caloric Contents. The fat content of lean meat is less than 6%; meat products, however, can contain fat levels as high as 50% (Table 44.1). For humans in western societies, the major fat intake from meat products is the backfat of the pig, which is present in many processed meat products; subcutaneous fat from ruminants is generally consumed less often (Raes and others 2004).

The major effort to reduce dietary fat focuses on frequently consumed foods having the highest fat percentages. Fat-reduction techniques are usually based on two main criteria: the utilization of leaner meat raw materials and the reduction of the fat density (dilution) by adding water and other ingredients. In the development of low-fat products, factors associated with meat raw materials, nonmeat ingredients (fat replacements or substitutes: proteins, carbohydrates, lipids), and manufacturing and preparation procedures should be taken into account (Keeton 1994; Jiménez-Colmenero 1996). Low-fat foods (e.g., meats) are functional foods (Thomson and others 1999) that are currently available in markets.

Population nutrient-intake goals for preventing diet-related chronic diseases (WHO 2003) take into account both the percentage of total fat energy and the relative contribution of different types of fatty acids. They are, therefore, a key consideration in designing the new composition of any product.

In general, in industrialized countries, the caloric intake from fat has been decreasing. The proportion of calories from fat is now roughly 36–40% (still quite far from the 30% recommended). Almost a fourth of the fat calories come from the consumption of meat and meat products (Sheard and others 1998; Chizzolini and others 1999). Fat contains twice the kilocalories per gram as proteins or carbohydrates (9 for fat versus 4 for protein and carbohydrates). Caloric intake is most frequently limited by reducing the proportion of fat. Depending on several factors, the calories can be reduced by nearly 50% with respect to normal-fat meat products (Wirth 1991; Sandrou and Arvanitoyannis 2000).

Reduction of Cholesterol Content. The amount of fat is not always directly related to cholesterol level (Table 44.1). In dry matter, the amount of cholesterol in lean beef, pork, lamb, and poultry tissue may be as much as twice that present in adipose tissue, but in wet matter, the cholesterol content of lean tissues is slightly lower than that of adipose tissue (Mandigo 1991). Therefore, reduction of the percentage of fat does not seem to be a suitable method of reducing cholesterol in meat products (Jiménez-Colmenero and others 2001).

It has even been suggested that if fat reduction is achieved by increasing the proportion of lean meat, it can actually increase the cholesterol level in the product (Mandigo 1991). The way to obtain meat products with less cholesterol is by replacing meat raw materials–fat and protein–with others that are devoid of cholesterol, particularly plant products or vegetable oils. The original composition of a number of meat products (ground beef, frankfurters, pork patties, and so on) has been modified by reducing animal fat and/or partially replacing it with vegetable oils (olive, corn, sunflower, soybean) and by incorporating different plant-based proteins (soy, corn, oat, wild rice, wheat gluten) or gums (Clarke 1997). This dilution method has made it possible to significantly reduce cholesterol content. For example, a cholesterol reduction of around 20% with respect to conventional meat products has been reported in low-fat ground-beef and pork sausages (Sandrou and Arvanitoyannis 2000). Replacement of 60% of the beef fat in frankfurters containing 29% fat with peanut oil reduced the cholesterol content by more than 35% (Marquez and others 1989). Using olive, cottonseed, and soy oils, Paneras and others (1998) obtained frankfurters (10% fat) with up to 59% less cholesterol than regular frankfurters containing 30% animal fat.

Reduction of Sodium Content. High salt intake has been related to high blood pressure, one of the major risk factors for CVD (Antonios and MacGregor 1997). The sodium intake in most developed countries greatly exceeds physiological requirements. The current level of salt consumption ranges between 6 and 20 g per day (Antonios and MacGregor 1997),

much higher than the <5 g per day recommended (WHO 2003). Although meat in itself is relatively low in salt, meat products with an elevated salt content present much higher sodium levels (Table 44.1). Over 80% of the current salt intake now comes from that added by food manufacturers (Antonios and MacGregor 1997), 20% being attributed to meat products (Wirth 1991). There is growing interest among consumers and processors in reducing the use of salt (minimizing sodium) in meat processing.

A variety of approaches for reducing the sodium content of meat products has been reported, including partial substitution of the sodium chloride added to meat products by other compounds (potassium and magnesium salts, phosphates, alginates, lactates, hydrolysates of collagen and peptides, and microbial transglutaminase). These substitutes can produce similar effects on sensory, technological, and microbiological properties. The extent to which salt levels can be limited depends on the type of meat product (Wirth 1991). In recent years, the salt content in meat products has been reduced to the point that there are now several commercially available salt-free meat products.

Reduction of Nitrites. Sodium nitrite is traditionally added to cured meat products to give meat products a characteristic color, to contribute to the aroma and taste, to inhibit the development of certain microorganisms (*Clostridium botulinum* and other food-borne pathogens), and as a potent antioxidant. In recent years, dietary nitrite has been associated with methemoglobinemia and the formation of nitrosamines, which are considered to be chemical agents with proven carcinogenic, mutagenic, and teratogenic activities. These compounds occur in a number of foods, including heat-cured meat products. They can form either in the food itself (depending on the heat-treatment conditions, salt, nitrite, and ascorbate concentrations, or pH) or in the stomach of the consumer (Pegg and Shahidi 1997).

As nitrosamine production depends on the residual nitrite level, the reduction of the latter will reduce the risk of these carcinogenic compounds forming. Another strategy is to use nitrosamine inhibitors. In fact, residual nitrite has been substantially reduced (up to 80%) in recent years. This change can be attributed to reduced nitrite addition, increased use of ascorbate, improvements in manufacturing processes and changes in composition (Cassens 1999). In coming years, Europe could increase the legal restrictions on nitrate and nitrite levels in meat processing.

REPLACING, ADDING AND INCREASING DIFFERENT COMPOUNDS IN MUSCLE FOOD PRODUCTS. A good way to increase the dietary intake of functional components is to incorporate them into common foods (Jiménez-Colmenero and others 2006). Meat product processing makes it possible to replace, add, or increase nonmeat ingredients (familiar or unfamiliar) with potential functional effects. Although replacement has focused mainly on lipids (and to a lesser extent on proteins), adding or increasing (fortified, enriched, or enhanced foods) involves a wide range of bioactive substances, unfolding myriad potential strategies (Table 44.2). Food fortification, which is considered to be one of the most successful approaches to functional foods (Wahlqvist and Wattanapenpaiboon 2002), plays an important role. The functional components have been used

1. In the form of specific preparations (of greater or lesser purity) utilized intentionally, and as constituents of certain nonmeat ingredients (extracts, flours, concentrates, homogenates, and so on); and

2. For different purposes (technological, sensory, nutritional, microbiological, economical) in the meat industry.

In reality, many of those nonmeat ingredients are of plant origin (oats, soybean, wheat, sunflower, rosemary, apple, mushroom, walnut), and their composition includes a wide range of beneficial components (phytochemicals) (Pennington 2002). Thus, their utilization as nonmeat ingredients implies the assurance of the presence of added bioactive components in many commercially processed meats. On the other hand, new possibilities arise as a consequence of migratory waves that introduce novel ethnic and regional specialties. These new foods and tastes enable the introduction of additional, more healthful products that may require expanding the array of components and technology.

The different types of functional components employed in the production of meat-based functional food are described below. Although they are presented individually here, in many cases the use of nonmeat ingredients containing complex mixtures of bioactive substances involves the coexistence of several types of interventions.

Modification of the Fatty Acid Profile. Meat product fatty acid composition can be modified by the formulation approach through the ingredients employed: meat raw material and nonmeat ingredients (Fernández-Ginés and others 2005). The first case entails the use of meat that, as a result of animal production practices (Section 44.3.1.1), presents a more favorable lipid profile in terms of improving the health status of the population (increased MUFA or ω-3 PUFA contents and reduced ω-6/ω-3 PUFA ratio). Several types of products have been prepared in this manner. Frankfurters and low-fat sausages containing high concentrations of MUFA have been made with meat raw materials from pigs fed on safflower, sunflower, and canola oils (St. John and others 1986; Shackelford and others 1990). Dry fermented sausages, cooked ham, and pork liver pâté with a healthier ω-6/ω-3 PUFA ratio have been manufactured using materials (backfat and meat) enriched in ω-3 PUFA, obtained from pigs fed on linseed-oil-enriched diets (Hoz and others 2004; Santos and others 2004; D'Arrigo and others 2004).

The second procedure consists in replacing part of the animal fat normally present in the product with another more suited to human needs – that is, with less saturated fatty acids and more MUFA (oleic acid) or PUFA and, moreover, without cholesterol. Simple fat replacement does not reduce the caloric content. Fish oils (ω-3 polyunsaturated oil) have been used for this purpose in low-fat frankfurters (Park and others 1989). Different vegetable oils (corn, cottonseed, palm, peanut, soybean, high-oleic acid sunflower, olive, linseed) have been used to replace animal fats (pork backfat or beef fat) in meat products such as frankfurters (Marquez and others 1989; Paneras and others 1998), ground beef patties (Liu and others 1991), and fermented sausages (Bloukas and others 1997; Muguerza and others 2002; Ansorena and Astiasaran 2004). Other types of nonmeat ingredients, such as walnut, have been utilized to produce healthier changes in the fatty acid profiles of frankfurters and restructured beefsteaks (Jiménez-Colmenero and others 2003). Walnuts display a high fat content (62–58%), being rich in MUFA (oleic acid) and PUFA (with linoleic and linolenic acids constituting 58% and 12%, respectively, of PUFA content). Besides modifications in the diet of the animals (Section 44.3.1.1), the interest in increasing the presence of CLA has led to its direct addition to meat products (Joo and others 2000).

On the other hand, the varying effects of fats (in part, depending on fatty acid composition) on satiety signals could be used in the development of fat-containing food (meat

products) that modulates satiety. Specific manipulations of fats (as well as proteins and carbohydrates) have the potential to act as functional foods for appetite control (Dye and Blundell 2002).

Factors associated with both the nature of the lipid material used for replacement and the type of product (degree of structural disintegration, fresh, cooked, fermented, and so on) condition its impact on the properties of the product and, consequently, the amount of meat fat that can be replaced.

Plant-based-Proteins. Several plant-based proteins have been used as ingredients in meat products essentially for technological (as binders and extenders), economical (reduce formulation cost), and compositional (nutritional and health) purposes. Examples of these protein additives are wheat flour, vital wheat gluten, soy flour, soy protein concentrate, soy protein isolate, textured soy protein, cottonseed flour, oat flour, corn germ meal, and rice flour, among others. Some of them have been used as fat replacements in low-fat meat products (Keeton 1994). Several of these nonmeat ingredients also contain bioactive compounds (Pennington 2002). For this reason, soy protein products have been widely studied. Owing to the presence, in addition to soy protein, of other compounds such as fiber, isoflavones, saponins, and so on they have been associated with health benefits such as reduced risk of heart disease, prevention of cancers and osteoporosis, and reduced menopausal symptoms (Hasler 1998). Other plant proteins also employed in meat products (sunflower, walnut) contain high proportions of arginine (a nitric oxide precursor) having a low lysine/arginine ratio. Many studies support the hypothesis that arginine or the low lysine/arginine ratio reduce arteriosclerosis and report beneficial effects on heart failure, blood pressure, and stroke (Feldman 2002).

Dietary Fiber. Regular fiber intake has a number of beneficial effects. Fiber is involved in regulating blood glucose and blood lipids, reducing the risk of diabetes, and preventing CVD, colon cancer, and regulating intestinal transit time. Europeans generally consume around 20 g/day, versus the more than 25 g/day recommended (WHO 2003). The incorporation of fiber into commonly consumed foods, like meat products, could help correct this deficiency. Fiber has been widely added to meat products, not only for its known physiological effects, but as a technological ingredient to improve water-binding properties, texture, and emulsion stability, helping to overcome the effects on the characteristics of meat products produced by composition changes (e.g., the fat reduction process).

Dietary fiber from oats, sugar beets, soy, rice, apples, peas, citrus fruits (lemon, orange), and so on, have been added to the formulation of several meat products such as ground meat and sausages (Keeton 1994; Kim and others 2000; Jiménez-Colmenero and others 2001; Fernández-López and others 2004; Fernández-Ginés and others 2005). Of special importance is inulin, a soluble dietary fiber composed of a blend of fructose polymers extracted from chicory that is being incorporated into numerous foods, including meat products (Pszczola 1998; Sloan 2000). The addition of antioxidant dietary fiber and chitosan to meat products also raises interesting possibilities.

Probiotics. Probiotics are living microbial food ingredients that are beneficial in such health concerns as gastrointestinal disorders, food allergies, inflammatory bowel diseases, and immune function. Examples of probiotics are lactic acid bacteria or bifidobacteria,

primarily of the *Lactobacillus* species (Työppönen and others 2003). The idea of using probiotic bacteria as fermenting agents in meat products is just beginning to develop.

Dry sausages are nonheated meat products that may be suitable carriers to deliver probiotics into the human gastrointestinal tract. Several of these products have been inoculated with *Bifidobacterium lactis, Lactobacillus casei, Lactobacillus paracasei*, and *Lactobacillus rhamnosus*. One essential aspect consists in ensuring that they survive the processing conditions (presence of nitrite, sodium chloride, or hostile environment) and the environment of the gastrointestinal tract. In order to achieve a health effect, the minimum daily intake of probiotic bacteria is estimated to be 10^{9-10} viable microbes. Thus, 10–100 g of dry sausage containing 10^8 viable microbes/g could be the minimum daily dose (Työppönen and others 2003). Dry sausages with an added probiotic culture are already being commercialized.

Tocopherols. Diet supplementation with vitamin E improves the oxidative capacity of meat (Morrissey and others 1998). Supplementation of meat products, such as reformed and restructured cured turkey products (Walsh and others 1998), cooked ham (Santos and others 2004), and dry fermented sausage (Hoz and others 2004) are also improved with vitamin E.

The antioxidant nutrient profile of meat products also has been improved by adding vitamin E during the manufacturing process, either as a specific preparation or as a component of nonmeat ingredients. Vitamin E has been added *in vitro* to several meat products (sausages, ham) and, in some cases, such as certain cured products, has been shown to decrease the production of nitrosamines (Gray and others 1982). Of the different plant sources that have been employed in the formulation of meat products, of particular interest are the wheat germ in frankfurters (Gnanasambandam and Zayas 1992) and walnut in restructured steak (Jiménez-Colmenero and others 2003). Although walnuts contain very low amounts of α-tocopherol (compared with other nuts), they have a high γ-tocopherol content, constituting an excellent dietary source of this vitamin.

Interestingly, γ-tocopherol exhibited a high antioxidant activity with potentially important physiological implications (Olmedilla and others 2006). Kim and others (2000) improved the oxidative stability of roast beef by incorporating the natural antioxidants (vitamin E vitamers and oryzanols) present in rice bran oil. Honey, which also has considerable antioxidant properties including α-tocopherol, ascorbic acid, catalase, and flavonoids, has been added to muscle food to protect against lipid oxidation (Pszczola 1998). At the present time, there are a number of commercially available vitamin-E-enriched meat products.

Carotenoids. In addition to nutritional strategies to incorporate carotenoids into intact muscle (Section 44.3.1.1), the use of carotenoids as an exogenous antioxidant additive in meat products with a certain structural disintegration has also been tested. Carotenoids are naturally present in different vegetables (Pennington 2002) employed as nonmeat ingredients in various processed meats (beef patties, restructured beef steak, frankfurters, and meat/liver loaves). Such is the case for tomato pulp (rich in lycopene) (Sánchez-Escalante and others 2003), carrot and sweet potato (rich in provitamin A) (Saleh and Ahmed 1998; Devatkal and others 2004), and spinach (rich in lutein and zeaxanthin) (Pizzocaro and others 1998).

Vitamin C. Ascorbic acid supplementation offers beneficial health effects as this vitamin produces a number of physiological effects that enhance immune function and help to prevent heart diseases and certain types of cancer (Johnston 2003). It is added to meat products either in the form of ascorbic acid or as part of certain nonmeat ingredients (mainly vegetables) employed in the formulation of meat products. Ascorbic acid has been incorporated into beef patties (Sánchez-Escalante and others 2001). Citrus byproducts have been added to cooked and dry-cured sausages as a source of ascorbic acid (Fernández-López and others 2004). The antioxidant properties of honey, resulting from its special composition (rich in ascorbic acid, among other substances), has led to its use as a protective agent (functional) against lipid oxidation in roasted chicken (Pszczola 1998) and cooked pork meat (O'Connell and others 2002).

Minerals. Certain meat products (e.g., sausages and turkey sausages) have been enriched with calcium (Harris 2000) and fluoridated salt. Their consumption is aimed at children to aid in bone and tooth development. On the other hand, the utilization of some nonmeat ingredients in processed meat can increase the levels of copper, magnesium, and manganese.

Plant Sterols. Plant sterols are present in most plants (Pennington 2002), some of which are used as nonmeat ingredients in processed meats. Structurally, plant sterols and stanols (saturated derivatives of sterols) are very similar to the cholesterol with which they compete during absorption in the intestinal tract. Stanol esters reduce the blood total cholesterol and low-density lipoproteins (LDL), without affecting the high-density lipoprotein (HDL) or triglyceride content. In 2000, the FDA authorized health claims for plant sterol and stanol esters based on evidence that they may help to reduce the risk of CVD. Several products have been developed in the form of foods typically consumed on a daily basis, including meat products such as frankfurters and broiler meatballs (Leino 2001).

Phytates. Phytate (myo-inositol hexaphosphate) is a natural antioxidant present in many plants (most cereals, nuts, and legumes). These phytochemicals also exhibits other health effects. Although phytate has antinutritional properties affecting mineral absorption, it also has anticarcinogenic effects, decreases kidney stones, lowers blood cholesterol in humans, and improves the glycemic index in human foods (Lee and others 1998). Phytates have been added to meat products; either in the form of commercial preparations (sodium phytate) in restructured beef (Lee and others 1998), or as components of several phytate-rich plants used as additives in different meat products; for example, rice fiber added to beef roast (Kim and others 2000).

Other Compounds. A number of other compounds are added to meat products during processing. They may be incorporated intentionally to achieve specific objectives or as components of one of the ingredients (soybean, nuts, onions) employed in the formulation. Examples of the intentional use are the addition of taurine, carnosine or spices to enhance lipid stability (Morrissey and others 1998; Sánchez-Escalante and others 2001) or limit the formation of mutagenic/carcinogenic heterocyclic amines (Gibis and others 1999). Other compounds are added unintentionally as ingredient components. Examples of unintentional use are isoflavones present in soy products (Sadler 2004) or the flavonols (which inhibit human platelet aggregation and prevent atherosclerosis) and allyl sulfides in onion (Pennington 2002; Fista and others 2004).

Consequences of Manufacturing Procedures on Meat Product Components Implicated in Human Health. In addition to the steps related to meat raw material and formulation, meat processing involves many other interventions that can modify the composition of the already formulated product. They can affect the content of (increasing or decreasing) some nutrients or other food components naturally present in food or the formation of others, as well as alter the microbial systems in functional foods (Knorr 1998). Some of these changes may have notable implications for human health. They can produce an increase in the density of some nutrients (e.g., due to cooking, drying) or the loss of others (due to cooking). On the other hand, meat and meat products undergo major chemical changes during processing (grinding, cooking, frying, smoking) that result in the formation of numerous compounds, many of which impart desirable characteristics to the food. Others, however, can have negative implications for human health. For example, although fermentation is intended to produce probiotics (Knorr 1998), in other cases, substances that possess potentially harmful biological properties may be formed, as is the case of nitrosamines, polycyclic aromatic hydrocarbons (PAH), heterocyclic amines, biogenic amines, and lipid oxidation products.

Cooking of cured meats may enhance nitrosamine formation. Polycyclic aromatic hydrocarbons result from the combustion of organic matter in the cooking and smoking of meat and meat products, as in many other foods. Their presence is determined by a number of factors, among them the composition of the product and the heat treatment applied. The importance of these hydrocarbons in certain meat products lies in the fact that some of them are carcinogenic (Hotchkiss and Parker 1990). Biogenic amines are compounds that are present in a large number of foods, including meat products. The biogenic amine concentration is conditioned by numerous factors, among them processing (curing) and preservation conditions. Consumption of foods with high concentrations of biogenic amines can cause migraine, headaches, gastric and intestinal problems, and pseudo-allergic responses, chiefly brought about by the toxic action of histamine and tyramine (Smith 1980).

Processing operations that disrupt the oxidation balance of skeletal muscle include particle size reduction (facilitates contact between oxygen and oxidizable lipids), cooking (causes a loss of antioxidant enzyme activity and release of protein-bound iron), and salting (increases the catalytic activity of iron and reduces antioxidant enzyme activity). These processing operations can dramatically increase lipid oxidation in muscle foods. Technological strategies to minimize the development of unhealthy products (Decker and Xu 1998) would help to reduce the risk of disease.

Recent research is disclosing the role of the proteins in meat as precursors of bioactive peptides, that is, fragments that are inactive within the precursor protein, but that can be released *in vivo* or *in vitro* by means of hydrolysis, and may exert different physiological functions in the organism. Arihara and others (1999) detected angiotensin-I-converting enzyme (ACE) inhibitory peptides in several commercial fermented meat products (e.g., cured loin of pork from Spain) and model sausages fermented with lactic acid bacteria. ACE plays an important physiological role in regulating blood pressure. Those authors suggested that ACE inhibitory activities can be generated from muscle proteins and could be utilized to develop functional foods (Arihara 2004). Some peptides from collagen hydrolysates inhibit fibrin polymerization and platelet formation, important elements of CVD (Garnier 2004).

Interestingly, CLA increases in foods that are cooked and/or otherwise processed (Arihara 2004). This is significant in view of the fact that many mutagens and carcinogens have been identified in cooked meats (Hasler 1998).

44.3.1.3 Distribution and Storage Conditions. From manufacture to consumer, meat products (like all other foods) must go through stages of distribution and storage, During this time changes can take place favoring the formation of harmful compounds such as those derived from lipid oxidation and biogenic amines. These changes can compromise the viability of probiotic strains (Knorr 1998).

44.3.1.4 Influence of Preparation and Consumption. Initially, in the selection of products, and later, during their preparation and consumption, consumers may modify food components that have implications for human health. Many foods, among them meats, undergo certain treatments before being consumed that markedly affect their composition.

The amount of fat ingested from a given product can vary widely depending on factors associated with the conditions of preparation and consumption. Cooking methods appear to be important. The fat content of cooked meat is sometimes higher than that of the corresponding raw meat, when expressed on a percentage basis, due to loss of large quantities of water during cooking. However, with respect to dietary fat intake, it is more meaningful to calculate the fat content on an absolute basis, based on an initial 100 g. Expressed thus, the cooking process reduces fat content in a proportion that depends on the type of product, its fat percentage, and the cooking method used: deep frying, grilling, roasting, boiling, or pan frying. In some cases, up to 25% or even 35% of the fat initially present can be lost during cooking (Sheard and others 1998). To make the most of this circumstance, pads have even been designed to absorb fat lost during the cooking process (microwave), minimizing the fat contact with food (Giese 1992). Even though the cholesterol content (per 100 g of meat) normally increases on a wet tissue basis after the cooking process (mainly due to loss of water), no changes in cholesterol content are observed in dry matter (Chizzolini and others 1999).

The amount of fat ingested also varies according to the manner in which it is separated from the meat during its preparation for cooking or when it is being eaten. The consumer can remove fat from meat, reducing the overall fat intake by variable amounts. Thus, trimming fat is a more effective means of reducing fat intake than simply reducing red meat consumption. The situation is rather different with meat products where the fat is present in a highly comminuted form and evenly distributed throughout the product (Sheard and others 1998).

44.3.2 Bioavailability Considerations

The presence of bioactive components in functional foods is not only to be considered. Other aspects that affect the bioavailability of the components are also relevant (Diplock and others 1999; Roberfroid 2000). In the different stages between meat production and its consumption, including processing, distribution, and storage, there are a number of factors that can affect the bioavailability of some components, modifying their functional effect and, thus, the role of meat products as functional foods.

44.3.2.1 Synergistic Effect of Absorption of Nutrients in Muscle Foods. It is believed that muscle foods possess intrinsic factors ("meat factors"), most of which have not been entirely delineated. These factors improve the bioavailability of a variety of nutrients. The most clearly established intrinsic effect of muscle food on a nutrient involves heme iron (Godber 1994). Meat enhances iron absorption from plant foods, so the presence of meat in a meal can double the amount of iron absorbed from other components of the meal.

Zinc absorption and retention are greater with high-meat diets, compared to low-meat or zinc-supplemented diets (Higgs 2000). The bioavailability of trace minerals such as zinc and copper is lower in plant foods than in muscle foods, which may even promote the bioavailability of calcium and/or magnesium given the apparent effect of these foods on bone mineralization. It has also been suggested that certain other nutrients, including vitamin B_6, folic acid, and niacin, are more readily available from animal sources than from plant sources (Godber 1994).

44.3.2.2 Effect of Processing on Bioavailability of Meat Product Components. A variety of processing procedures used in the manufacture of meat products, their distribution and storage, and even consumer practices can have an impact on the efficiency of absorption of some nutrients, and interactions between different food components can affect the bioavailability of some of them. These interactions can take place during meat processing involving certain nonmeat ingredients, such as fiber or phytic acid. These ingredients bind a number of minerals, which decreases mineral bioavailability (Godber 1994; Kim and others 2000). However, meat provides an assured source of iron, as heme-iron is unaffected by the numerous inhibitors of iron absorption such as phytate (Higgs 2000). In addition to the formation of several compounds that have negative effects on health, lipid oxidation also leads to the loss of nutrients susceptible to oxidative degradation, as is the case for fat-soluble vitamins A and E and certain water-soluble vitamins, including thiamin and folic acid (Godber 1994).

Studies have shown that, depending on different factors, cooking (whether by the meat processor or the consumer) can reduce the bioavailability of some bioactive compounds in muscle tissue, such as taurine, carnosine, coenzyme Q_{10} (ubiquinone), and creatine. These compounds are associated with several health benefits. For example, carnosine and coenzyme Q_{10} have antioxidant properties and taurine presents antioxidant activity and protects against exercise-induced muscle damage, but, under certain circumstances, creatine contributes to the formation of muscle mass (Purchas and others 2003). Losses of carnitine during storage and cooking of meat have been reported. This small molecule is essential for fat metabolism as a vitamin-like material and has a relation with various diseases (Wakamatsu 1999).

44.4 CONCLUSIONS

The meat industry is changing rapidly, impelled by changes in food technology and demand. Functional foods pertain to a category of products that clearly respond to consumer preferences, and a good way to increase functional components into the diet is to incorporate them into common foods. As meat is one of those most widely consumed, it would appear to be an excellent vehicle for functional component delivery.

Although the idea of functional foods is more closely associated with other foods, meat and meat products contain a number of compounds (naturally present or added) that confer the properties of functional food to them. A number of technological and biotechnological strategies make it possible to modify the content and/or bioavailability of functional components from different sources, a circumstance that raises considerable expectations concerning the possibility of generating meat products better adapted to the specific needs of large sectors of society. Meat-based functional foods would help to integrate meat and meat products into healthful diets.

Most of the studies on meat-based functional foods (like those dealing with most functional foods) focus mainly on production systems, concentrating on the presence of one or more functional components. One aspect that may place in doubt the benefits of some of these practices is modifications affecting the bioactive components of meat products that may decrease the quantitative importance at a dietary level. However, dietary diversification and modification is one of the intervention strategies recommended to increase the intake of certain food components and reduce the occurrence of micronutrient malnutrition (Gibson and Hortz 2001).

Although essential, there are few studies designed to elucidate the behavior and biological activity of the different functional components of meat products (or other functional foods) in terms of the processing conditions, as well as their functional impact on the organism. In this respect, it is crucial to assess the possible changes in the bioavailability of the functional components after the many stages of preparation and preservation of foods for human consumption.

It is also of the utmost importance to clearly establish the optimal levels of most of the biologically active components in order to ensure that their effects are truly beneficial at the doses and under the conditions in which they are consumed (Hasler 1998). Foods can be regarded as functional if they can be demonstrated to affect beneficially one or more target functions in the body, beyond an adequate nutritional effect, in a way that is relevant to an improved state of health and well-being and/or reduction of risk of disease. In-depth studies that provide scientific evidence of the functional effects of meat-based functional foods are indispensable.

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45

Gluten-Free Cereal Products as a Functional Food

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45.1 Introduction 1018
45.2 Celiac Disease 1018
45.2.1 Epidemology 1018
45.2.2 The Iceberg Model 1019
45.2.3 Possible Causes 1019
45.2.4 Consequences of Gluten Intolerance 1020
45.2.5 Treatment and Dietary Requirements 1020
45.2.6 Codex Alimentarius 1021
45.3 Development of Gluten-Free Cereal Products 1021
45.3.1 Dietary Fiber 1022
45.3.2 Sourdough 1023
45.3.3 Pseudocereals 1023
45.3.4 Sorghum 1025
45.3.5 Oats 1026
45.3.6 Transglutaminase 1027
45.3.7 Hydrocolloids 1027
45.3.8 Protein Sources in Gluten-Free Breads 1029
45.3.9 Starch Sources in Gluten-Free Bread 1030
45.4 Conclusion 1030
References 1030

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45.1 INTRODUCTION

The demand for gluten-free foods is constantly rising, due to the increased number of consumers suffering from food intolerances or a disorder referred to as celiac disease. The majority of the gluten-free products on the market are of very poor quality and, due to the unique properties of gluten, in the production of cereal products it is a great challenge for the food scientist to create products comparable to their wheat counterparts.

45.2 CELIAC DISEASE

In recent years there has been a slow and steady increase in consumer interest in wheat-free foods, driven in part by an increasing awareness of the relatively unfamiliar condition known as celiac disease (Lovis 2003). Celiac disease, also known as nontropical sprue, celiac spru, idiopathic steatorrhoea, primary malabsorption, Gee–Herter disease, gluten-induced enteropathy, and adult celiac disease (Cooke and Asquith 1974), is a gluten-sensitive entropy characterized by the damage of the small-intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamins) of barley and rye in genetically susceptible people (Murray 1999; Fasano and Catassi 2001; Farrell and Kelly 2002). Table 45.1 (Feighery 1999) outlines the most commonly encountered reactions to gluten by sufferers of celiac disease.

45.2.1 Epidemology

Celiac disease was once thought to be a rare condition and confined to childhood (Green and Jabri 2003), with a uniform clinical presentation of weight loss and diarrhea. Due to improved diagnostic procedures (antigliadin antibody serological tests) and increased

TABLE 45.1 Symptoms (and Related Signs) of Celiac Disease.

Infancy (0–2 years)
Diarrhea (miserable, pale)
Abdominal distension (enlarged abdomen)
Failure to thrive (low weight, lack of fat, hair thinning)
Anorexia, vomiting
Psychomotor impairment (muscle wasting)
Childhood
Diarrhea or constipation
Anemia
Loss of appetite (short stature, osteoporosis)
Adulthood
Diarrhea or constipation
Anemia
Aphthous ulcers, sore tongue and mouth (mouth ulcers, glossitis, stomatitis)
Dyspepsia, abdominal pain, bloating (weight loss)
Fatigue, infertility, neuropsychiatric symptoms (anxiety, depression)
Bone pain (osteoporosis)
Weakness (mypoathy, neutopathy)

Source: Feighery (1999).

TABLE 45.2 Prevalence of Celiac Disease Based on Clinical Diagnosis or Screening Data.

Geographic	Prevalence on Clinical Diagnosis	Prevalence on Screening Data
Denmark	1 : 10,000	1 : 500
Finland	1 : 1000	1 : 130
Germany	1 : 2300	1 : 500
Italy	1 : 1000	1 : 184
Netherlands	1 : 4500	1 : 198
Norway	1 : 675	1 : 250
Sweden	1 : 330	1 : 190
United Kingdom	1 : 300	1 : 112
United States	1 : 10,000	1 : 111
Worldwide Average	1 : 3345	1 : 266

Source: Fasano and Catassi (2001).

awareness of celiac disease, the rate of diagnosis has significantly increased. In Europe, the prevalence of celiac disease is estimated to be 1 in 300 to 1 in 500 persons, but recent population-based screening studies suggest that the prevalence may be as high as 1 in 100 (Mustalahti and others 2002). Table 45.2 (Fasano and Catassi 2001) illustrates the prevalence of celiac disease based on clinical diagnosis and screening data. However, a review by Farrell and Kelly (2002) suggests that the true prevalence of celiac sprue is difficult to ascertain, because many patients have atypical symptoms or none at all. Celiac disease is more frequent throughout Europe than previously thought. From Table 45.2, it is evident that the prevalence of celiac disease is as frequent in the United States as in Europe (Fasano and Catassi 2001). From results obtained from screening data, Fasano and Catassi (2001) concluded that celiac disease is one of the most frequent genetically based diseases (Catassi and others 1994; Ascher and others 1991).

45.2.2 The Iceberg Model

The iceberg is a common model (Fig. 45.1) used to explain the epidemiology of celiac disease (Visakorpi and others 1996). The few cases that have been properly clinically diagnosed with the classical symptoms of chronic diarrhea, unexplained iron deficiency, failure to thrive, and the presence of a flat mucosa form the small top of the iceberg (Fasano and Catassi 2001). Patients who have been recently diagnosed and who are now living with gluten-free diets and show a normal mucosa form the lower part of the tip. The ratio of diagnosed to undiagnosed cases gives the so-called "waterline". A relatively large group of "silent" cases are situated below the waterline. These cases have not been identified and show a small bowel mucosa lesion but may remain undiagnosed because the clinical symptoms are lacking (Feighery 1999). The bottom end of the iceberg consists of a small group of people with latent celiac disease. These patients show a normal musosa while ingesting gluten. They have the potential to develop the disease at any stage in their life.

45.2.3 Possible Causes

According to Murray (1999), celiac disease is the end result of three processes that culminate in intestinal mucosal damage: genetic predisposition, environmental factors, and immunologically based inflammation.

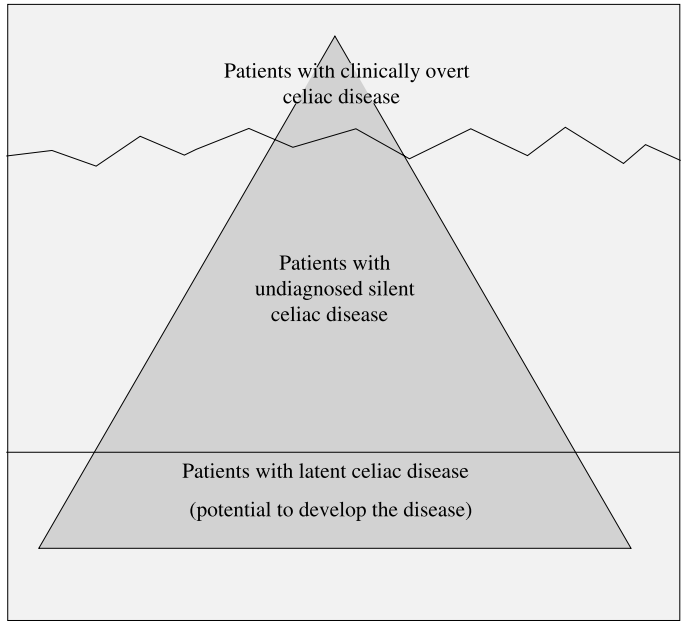

Figure 45.1 *Iceberg model depicting prevalence of celiac disease (Adapted from Feighery 1999).*

45.2.4 Consequences of Gluten Intolerance

With prolonged exposure to gluten in the diet, the consequences of celiac disease both internally and externally increase over time. The internal effects are the continued presence of a flat intestinal mucosa, quite often followed by a reduction in enzyme activity and hence lack of vitamins and minerals, which in turn may lead to different deficiencies. The external effects include dermatitis herpetiformis or the appearance of pale skin, dry hair roots, or abdominal distention, especially in children younger than two years of age.

45.2.5 Treatment and Dietary Requirements

The only way that celiac disease can be treated is the total lifelong avoidance of gluten ingestion (Fasano and Catassi 2001; Mowat 2003). The removal of gluten from the diet results in an improvement of the clinical symptoms and the mucosal lesions (Hansson 1999). Patients with celiac disease are unable to consume some of the most common products on the market today, namely breads, baked goods, and other food products made with wheat flour (Lovis 2003). Hidden ingredients, for example, byproducts or processed foods that contain wheat and gluten-derivates as thickeners and fillers, must also be avoided, These include hamburgers, salad dressings, cream sauces, dried soup mixes or canned soups, and processed cheese. Medications may include wheat proteins as binders and therefore must also be excluded. Other cereals such as rye, barley, malt, kamut, einkorn, dinkel, and spelt are prohibited. As celiac disease can in fact result in lactose intolerance due to the lack of lactase production (Murray 1999), persons with lactose intolerance due to celiac disease must avoid cow's milk.

45.2.6 Codex Alimentarius

The Codex Standard for gluten-free foods was adopted by the Codex Alimentarius Commission of the World Health Organization (WHO) and by the Food and Agriculture Organization (FAO) in 1976. In 1981 and 2000, draft revised standards stated that so-called gluten-free foods are described as

1. Consisting of, or made only from ingredients that do not contain any prolamins from wheat or all *Triticum* species such as spelt, kamut, or durum wheat, rye, barley, oats or their crossbred varieties with a gluten level not exceeding 20 ppm; or
2. Consisting of ingredients from wheat, rye, barley, oats, spelt or their crossbred varieties, which have been rendered gluten-free, with a gluten level not exceeding 200 ppm; or
3. Any mixture of two ingredients as mentioned in (1) and (2) with a level not exceeding 200 ppm.

In this context the WHO/FAO standard gluten was defined as a protein fraction from wheat, rye, barley, oats or their crossbred varieties (e.g., Triticale) and derivatives thereof, to which some persons are intolerant and which is insoluble in water and 0.5 M NaCl. Prolamins are defined as the fraction from gluten that can be extracted by 40–70% aqueous ethanol. The prolamin from wheat is gliadin, from rye is secalin, from barley is hordein, and from oats is avenin. The prolamin content of gluten is generally taken as 50%. In the United States and Canada, the gluten-free diet is devoid of any gluten, and is based on naturally gluten-free ingredients such as rice. However, in the United Kingdom and most European countries, products labelled as being gluten-free may still contain an amount of wheat starch.

45.3 DEVELOPMENT OF GLUTEN-FREE CEREAL PRODUCTS

In view of the fact that gluten is responsible for the viscoelastic properties in wheat bread, its replacement is one of the biggest challenges when developing gluten-free cereal products. The structure of gluten is complex, and is stabilized by intermolecular disulfhide, hydrogen, and hydrophobic bonds. The gluten proteins present in the wheat flour are embedded in the flour particles along with the other flour components, mainly starch granules as indicated in Figure 45.2. This micrograph shows large starch granules (dark gray areas) embedded in a continuous protein network (white areas). Gluten also contributes to the texture and crumb structure of the final baked product (Faubion and Hoseney 1990; Stear 1990).

A marketing review by Arendt and others (2002), reported that most of the gluten-free products were of inferior quality and very often showed off-flavours. The structure of the products was mostly crumbly and very dry. According to Gujral and others (2003a), gluten-free breads are usually characterized by deficient quality characteristics as compared to wheat breads. Problems related to volume and crumb texture are associated with gluten-free breads, even when rice flour is used, which seems to be the best raw material for this type of bread (Gujral and others 2003a). Several studies have been conducted by O'Brien and others (2002), Gallagher and others (2002), Schober and others (2003), and Moore and others (2004a,b,c), in which novel ingredients such

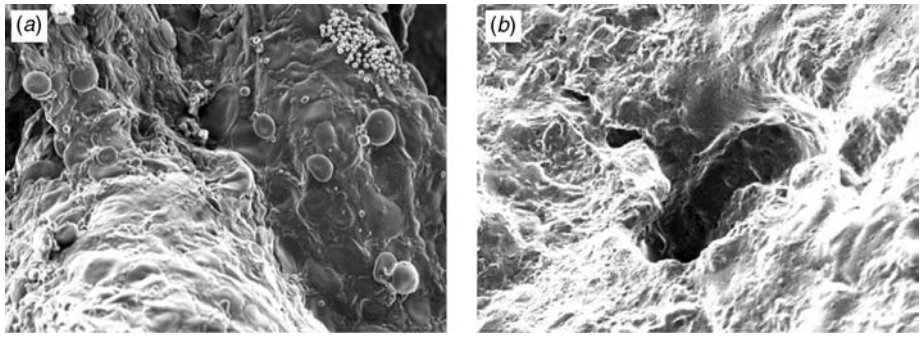

Figure 45.2 *Scanning electron micrographs of wheat bread (a) and gluten-free bread (b). Magnification* $\times$ *430.*

as dairy powders, sorghum, rice, starches, pseudocereals, and so on, in combination with hydrocolloids replaced gluten. Gluten-free products, for instance, must not contain wheat, rye, and barley or the protein-containing constituents of these (Paulus 1986). Gluten-free breads require a different technology. The properties of the dough are more fluid than wheat doughs and closer in viscosity to cake batters (Cauvain 1998; Moore and others 2004a) due to the lack of a gluten network. These batter-type doughs have similar handling properties to cake batters rather than typical wheat doughs. Furthermore, gas holding is more difficult, and the use of gums, stabilizers, and pregelatinized starch has been suggested to provide gas occlusion and stabilizing mechanisms (Cauvain 1998; Satin 1998).

45.3.1 Dietary Fiber

Cereals are an important source of dietary fiber, contributing to about 50% of the fiber, intake in western countries. The role of dietary fiber in providing roughage and bulk, and in contributing to a healthy intestine, has long been recognized. Diets that contain moderate quantities of cereal grains, fruits, and vegetables are likely to provide sufficient fiber. Due to the fact that gluten-free products are generally not enriched/fortified, and are frequently made from refined flour or starch, they may not contain the same levels of nutrients as the gluten-containing counterparts they are intended to replace. Therefore, uncertainty still exists as to whether celiac patients living on a gluten-free diet are ensured a nutritionally balanced diet, in particular regarding dietary fiber intake. Grehn and others (2002) screened the intake of nutrients and foods of 49 adults diagnosed with celiac disease and following a gluten-free diet. They were found to have a lower intake of fiber when compared to a control group of people on a normal diet. Similarly, Lohiniemi and others (2000) found that the average fiber consumption amongst celiacs in Sweden was lower than recommended. In their studies with celiac adolescents, Mariani and others (1998) concluded that adherence to a strict gluten-free diet worsens the already nutritionally unbalanced diet of adolescents. (Dietary levels of nutrients and fiber were found to be low.) Similar findings were revealed by Thompson (2000).

The enrichment of gluten-free baked products with dietary fibers has, therefore, been a topic of research for various teams of technologists. Studies have shown that the addition of high-fiber foods can give texture, gelling, thickening, emulsifying, and stabilizing properties to certain foods (Dreher 1987; Sharma 1981). Inulin is a nondigestible

polysaccharide that is classified as a dietary fiber. Inulin, a fructooligosaccharide, is used either as a macronutrient substitute or as a supplement added in foods mainly for its properties (Zimeri and Kokini 2003). Inulin consists of fructose molecules linked by β (2-1). It also acts as a prebiotic by stimulating the growth of "healthy" bacteria in the colon (Gibson and Roberfroid 1995). When added to wheat bread, it improves loaf volume and sliceability, increases dough stability, and produces a uniform and finely grained crumb texture (Anon 1999). Gallagher and others (2002) incorporated inulin (8% inclusion level) into a wheat-starch-based gluten-free formulation. The dietary fiber content of the bread increased from 1.4 (control) to 7.5% (control + inulin). The overall quality of the gluten-free bread was also improved by the addition of inulin.

45.3.2 Sourdough

Sourdough fermentation has a well-established role in improving the flavor and structure of bread. However, the significant potential of sourdough fermentation to improve the nutritional properties of rye, oat, and wheat products has gained much less attention, although interest is currently increasing. Sourdough fermentation improves the nutritional value of cereal products in a number of ways: it can improve texture and palatability of whole grain, fiber-rich, or gluten-free products, stabilize or increase levels of various bioactive compounds, retard starch bioavailability (lower glycemic index products), and improve mineral bioavailability. Many new interesting applications for sourdough remain still to be explored, such as the use of prebiotic starter cultures or production of totally new types of bioactive compounds (Katina and others 2004).

Gluten-free breads tend to show quick staling and a flat aroma. It has been reported for wheat bread that both of these disadvantages can be overcome by the application of sourdough (Moore and others 2004b). Moore and others (2004b) carried out a study in which they evaluated over a five-day storage period the influence of sourdoughs made from different strains of lactic acid bacteria on the quality of gluten-free bread. In this study, sourdough-containing breads were compared to a nonacidified control and a chemically acidified control. Changes in dough structure could not only be detected by small-deformation viscoelastic measurements but also by confocal laser-scanning microscopy (Fig. 45.3). The protein fraction of the gluten-free sourdough was degraded over time (Fig. 45.3); this process was, however, far less obvious in a gluten-free system than with gluten isolated from wheat-based sourdough (Fig. 45.3). When the sourdoughs were incorporated at a 20% level into the gluten-free batter, no significant differences were observed in the structure (Fig. 45.3), which is not the case in wheat dough containing 20% sourdough (Fig. 45.3). These authors concluded that with the application of sourdough, the onset of staling was delayed, which led to an improvement in the gluten-free bread.

45.3.3 Pseudocereals

Buckwheat, amaranth, and quinoa, due to their unique chemical structures, are classified as pseudocereals. There are two major subclasses of flowering plants: monocots (one seed leaf) and dicots (two seed leaves). Wheat, rye, and barley are monocots, whereas buckwheat, amaranth, and quinoa are dicots and very distantly related to grains in the monocot subclass (Kasarda 1994, 1997, 2001). The unique protein structure and amino acid composition imply that buckwheat might be a very valuable functional food resource and will benefit people in treating some chronic diseases such as diabetes and

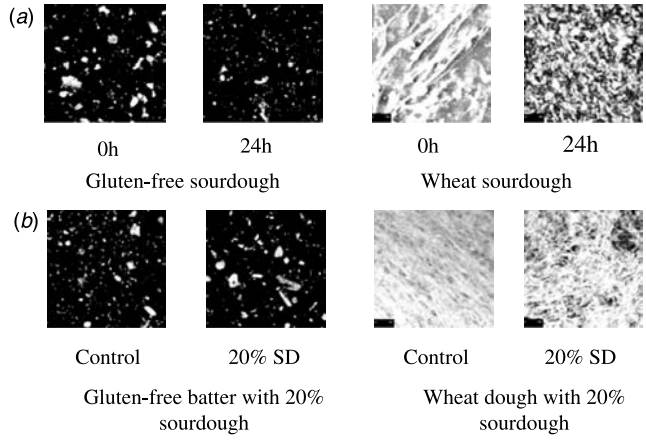

Figure 45.3 *Confocal laser-scanning micrographs of (a) gluten-free sourdough and wheat sourdough at 0 h and after 24 h (magnification bar corresponds to 50 μm) and (b) gluten-free batter with 20% sourdough and wheat dough with 20% sourdough. (Adapted form Clarke and others 2004.)*

hypertensionas well as other cardiovascular diseases (Li and Zhang 2001). The addition of buckwheat, amaranth, and quinoa would add nutritional value to the diet of a person with celiac disease. Many gluten-free cereal foods (e.g., bread, pasta, cold cereal) are made from refined flour and/or starch and most are not enriched with iron and B vitamins (Thompson 1999). As a result, a gluten-free diet may contain inadequate amounts of fiber, iron, thiamine, riboflavin, niacin, and folate (Thompson 1999, 2000). Buckwheat, amaranth, and quinoa are all good sources of fiber and iron. In addition, the riboflavin content of quinoa, and the niacin content of buckwheat flour compare favorably with that of enriched wheat flour (U.S. Department of Agriculture 2000).

Moore and others (2004a) produced a high-quality gluten-free bread (Fig. 45.4c) containing buckwheat. These authors reported that bran particles from brown rice flour or buckwheat contain high concentrations of fiber and swell extensively. Water absorption for the bread formulation increased upon the addition of buckwheat, but at the expense of volume. Even though these authors concluded that this particular bread was firmer in texture, the staling rate was lower in comparison to the starch-based commercial gluten-free bread (Fig. 45.4). Also, with the addition of buckwheat to gluten-free bread, the nutritional properties of the bread were enhanced. Furthermore it may also be used in the production of gluten-free beers, especially as a popular adjunct (Maccagnan and others 2004). Wijngaard and others (2004) carried out a study on the impact of germination temperature on the quality of buckwheat malt using both hulled and unhulled buckwheat. Their objective was to produce gluten-free beer using buckwheat as a replacement for barley. These authors concluded that buckwheat malt has great potential as a brewing ingredient.

Several studies have been carried out by Lorenz (1981) on the physico-chemical properties of the amaranth starch. This author found that the water-binding capacity of the amaranth starch was higher than that of wheat starch, but the swelling power values for the amaranth starch were lower than those of wheat starch and solubility values higher. Lorenz (1981) suggested that the small size of the amaranth starch granules was the possible reason for the observed differences. Gambus and others (2002) replaced cornstarch with amaranthus flour to enhance the protein and fiber contents of gluten-free breads. At a 10% replacement level, protein and fiber levels increased by 32 and 152%

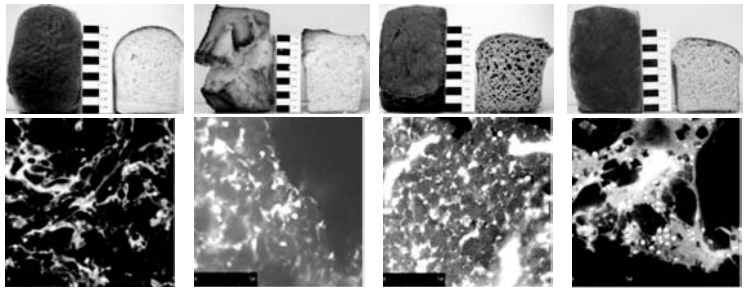

Figure 45.4 *Wheat bread control and gluten-free breads (bread from commercial gluten-free flour, nondairy recipe, dairy recipe. (a) Outer appearance of the breads and microscopical structure as detected by confocal laser-scanning microscopy (magnification bar corresponds to 50 μm); (b) Loaf specific volume (mL/g) values for wheat bread (W), commercial gluten-free bread mix (C), nondiary gluten-free (ND) and dairy gluten-free (D) breads, and (c) Crumb hardness values for wheat bread (W), commercial gluten-free bread mix (C), nondiary gluten-free (ND) and dairy gluten-free (D) breads over a 5-day storage period labeled with a common lower case letter within the same bread type are not significantly different ($p < 0.01$). Mean values for a given storage time labeled with a common upper case letter are not significantly different ($p < 0.01$).*

respectively, but sensory quality was unaffected. Tosi and others (1996) described the use of amaranth in gluten-free products. They formulated a gluten-free mix using wholemeal amaranthus flour. Taylor and Parker (2002) discussed the application of quinoa as a novel application in the production of enriched gluten-free bakery goods. Both quinoa and amaranth are pseudocereals, which have a high nutritional value and only recently are being utilized as novel/functional ingredients.

45.3.4 Sorghum

Sorghum (*Sorghum Bicolour (L.) Moench*) is an important cereal grain and belongs to the grass family *Graminae* and tribe *andropoggonae*. Like other cereal grains, the primary component of sorghum is carbohydrate in the form of starch (Rooney and Waniska 2000). Sorghum has a similar chemical composition to maize. However, sorghum is often reported to have a slightly lower protein and starch digestibility when compared to maize. This is especially true for cooked sorghum and has been attributed to increased cross-linking of the proteins during cooking (Hamaker and Bugusu 2003; Schober and others 2006). The average protein content of sorghum is 11–12% (Hoseney and others 1981). Sorghum is often recommended as a safe food for celiac patients, because it is more closely related to maize than to wheat, rye, and barley (Kasarda 2001). Sorghum might therefore provide a good basis for gluten-free bread. However, the majority of studies dealing with leavened loaf breads containing sorghum have focused on composite breads from wheat and sorghum, in which a maximum of only 30% low-tannin sorghum is regarded as acceptable (Munck 1995). Although such breads have been found acceptable by consumers (Carson and others 2000), they are inappropriate for celiac patients.

Recent research on sorghum has shown that it has tremendous potential as a "functional" food (Schober and others 2006). Specific types of sorghum have a very high content of tannins, which are polyphenolic compounds. Tannins are powerful antioxidants and are found in tea, chocolate, and wine. The potential health benefits of tannins for humans have recently been

reviewed by Awika and Rooney (2004). These authors reviewed the health benefits of a number of other phytochemicals in sorghum, including phenolic acids, anthocyanins, phytosterols, and policosanols. Phytochemicals found in sorghum were reported to have a potential impact against cancer and obesity as well as promoting cardiac health. Policosanols are found in the wax surrounding the sorghum kernel. These compounds and others in the wax, namely long-chain fatty acids, may have important cardiac health benefits such as lowering cholesterol (Hwang and others 2004; Hargrove and others 2004).

Only a limited number of studies have addressed such wheat-free sorghum breads and most have used relatively complex recipes incorporating xanthan gum (Satin 1988), carboxymethyl cellulose, and skimmed milk powder (Cauvain 1998), egg (Keregero and Mtebe 1994; Cauvain 1998), or rye pentosans (Casier and others 1997). Schober and others (2004) investigated the impact of different sorghum varieties on the quality of gluten-free bread. Significant differences were observed between the various sorghum varieties. The addition of skim milk powder and xanthan gum led to an improvement of the gluten-free bread quality. The formulation used in that paper was mainly based on sorghum and was very simple.

45.3.5 Oats

There is much debate on whether or not oats may be used in the production of gluten-free products. A review by Thompson (2003) reports that most adults with celiac disease can consume moderate amounts of uncontaminated oats without causing damage to the intestinal mucosa. Studies have been carried out by Janatuinen and others (1995), Srinivasan and others (1996), Hardman and others (1997), in which they concluded that oats cereal is neither toxic nor immunogenic in celiac disease. According to Hardman and others (1997), oats belong to a different tribe, Aveneae, than that of wheat, rye, and barley. Oat prolamin (avenin) has a lower proline content than the prolamins present in wheat, rye, and barley (gliadin, secalin, and hordein, respectively). The sequence glutamine-glutamine-glutamine-proline-phenylalanine-proline is found in prolamins of wheat, rye, and barley, but so far has not been found in oats (Janatuinen and others 1995; Srinivasan and others 1996; Hardman and others 1997). The ability of oat prolamins to induce an immune response in intestinal biopsy specimens *in vitro* as well as the cross-reactivity of monoclonal antibodies to enterotoxic wheat peptides with oat peptides are some of the reasons cited for continued caution (Parnell and others 1998). Furthermore the risk of contamination of oats with wheat, rye, or barley before reaching the consumer is considered high. The Gluten Intolerance Group, Celiac Disease Foundation (Celiac Disease Foundation/Gluten Intolerance Group 2000), Celiac Sprue Association (2001), and the American Dietetic Association (Inman-Felton 1999; American Dietetic Association and Dieticians of Canada 2000) all advise against the use of oats, but the Finnish Celiac Society considers them safe for consumption by adults (The Finnish Celiac Society 2001) as does the Celiac Society in the United Kingdom (in moderate amounts) (The Celiac Society 2001).

Oats contain many beneficial properties. According to Oomah and Mazza (1999), oat β-glucan is involved in carbohydrate and lipid metabolism. It has beneficial effects in the treatment of diabetes, and lowers serum cholesterol. Oats also contain a multitude of other compounds, such as phytates, phenolics, vitamins, and minerals, which confer other physiological benefits. These include powerful antioxidants and are utilized by the pharmaceutical and cosmetics industries. Furthermore, high consumption of oat meal, oat bran, or oat flour can reduce the risk of coronary heart disease.

45.3.6 Transglutaminase

Transglutaminase (TG: protein-glutamine γ-glutamyltrans-ferase, EC 2.3.2.13) is a promising enzyme for the food industry. Transglutaminase is an enzyme capable of catalysing acyl-transfer reactions, introducing covalent crosslinks between proteins (Nonaka and others 1989) as well as peptides and various primary amines. Crosslinking occurs when the ε-amino groups of lysine residues in proteins act as an acyl-receptor, and ε-(γ-Glu)Lys bonds (isopeptide bonds) are formed both intra- and intermolecularly (Ando and others 1989).

Transglutaminase is a highly functional enzyme and may be obtained from a range of different sources. These include animal tissue, fish, plant, and microorganisms (Kuraishi and others 1996). According to Aeschlimann and Thomaz (1999), transglutaminase can be connected with several biological phenomena such as blood clotting, wound healing, as well as with a number of pathologies including neurodegenerative disease, cancer metastasis, and celiac disease. The transglutaminases used in baking applications are aminotransferases and are obtained from microbial cultures. Transglutaminase has the ability to link proteins of different origins: casein and albumin from milk, animal protein from eggs and meat, soya protein and wheat protein. Transglutaminase has been mainly used in the dairy and meat industry to improve water-holding capacity.

Moore and others (2004c) reported that microbial transglutaminase can be used to create a network-like structure in gluten-free bread (Fig. 45.5) and therefore has the potential to improve the quality of gluten-free bread. However, the use of transglutaminase in gluten-free bread is very much dependent on the protein source used in the bread formulation.

45.3.7 Hydrocolloids

Hydrocolloids have been essential ingredients in the formulation of gluten-free cereal products. Hydrocolloids or gums are substances consisting of hydrophilic long-chain, high-molecular-weight molecules, usually with colloidal properties, which in water-based systems produce gels, that is, highly viscous suspensions or solutions with low dry-substance content (Hoefler 2004). They are derived from seeds, fruits, plant extracts, seaweeds, and micro organisms, being of polysaccharide or protein nature (Norton and Foster 2002). They are generally polysaccharides, but gelatin (a protein) is included because its functionality is very similar to that of the polysaccharide-based gums (Hoefler 2004). Hydrocolloids or gums serve two basic functions in food systems: They

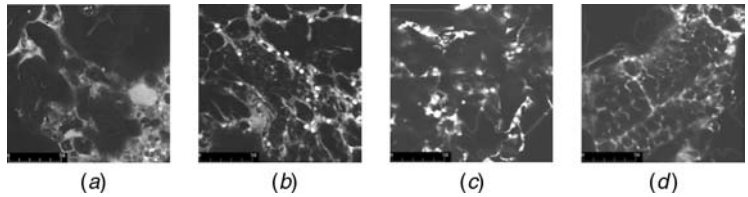

Figure 45.5 *Confocal laser-scanning micrographs of gluten-free breads, Egg 0 unit TG/g Protein (control) (a), Egg 1 unit TG/g Protein (b) SMP 0 unit TG/g Protein (control) (c), and SMP 10 unit TG/g Protein (d). The magnification bar corresponds to 50 μm for the objective x63.*

stabilize the product, and they affect the texture of the product, and are widely used in the food industry for these functional properties. These functional properties lead to the improvement of food texture, they retard starch retrogradation, increase moisture retention, and extend the overall quality of the product over time (Armero and Collar 1996a,b; Davidou and others 1996).

Hydrocolloids have been found to affect dough rheological performance, as they mimic the viscoelastic properties of gluten in bread doughs (Smith 1971; Toufeili and others 1994; Collar and others 1998) and also swelling, gelatinization, pasting properties, and staling of starch. According to Toufeili and others (1994) and Guarda and others (2004), the utilization of polymeric substances such as xanthan gum or hydroxypropylmethylcellulose (HPMC) are required for the production of gluten-free breads. The lack of a gluten network determines the properties of the gluten-free dough, which is more fluid than wheat doughs and closer in viscosity to cake batters (Cauvain 1998; Moore and others 2004a) and thus has also to be handled in a similar manner to cake batters rather than doughs. Most gluten-free breads are formulated using gluten-free starches and require the addition of hydrocolloids to provide structure and gas retaining properties in the dough (Ylimaki and others 1998; Moore and others 2004a). Schwarzlaff and others (1996) used combinations of guar gum and locust bean gum to partially replace flour in bread. They found that the introduction of guar gum resulted in a crumb structure with a more even cell size distribution, and locust bean gum inclusion increased the height of the bread loaves; both gums retarded bread staling. Optimum levels for locust bean gum and guar gum were 2–4%. For gluten-free bread production, the most commonly used hydrocolloids are pectin, guar gum, xanthan gum, and locust bean gum (Smith 1971; Igoe 1982; Okubo and others 1994). Kang and others (1997) carried out studies in which they looked at the effect of various hydrocolloids on a rice bread formulation. The inclusion of many gum types such as hydroxypropylmethylcellulose (HPMC), locust bean gum, guar gum, carageenan, xanthan gum, and agar gave successful formation of rice bread, and HPMC gave optimum volume expansion (Kang and others 1997).

Konjac gum is a polysaccharide that has the ability to form cohesive, tough films that remain stable in hot and cold systems (Tye 1991). Konjac is therefore considered a useful hydrocolloid in the production of gluten-free bread. Moore and others (2004a) produced good quality gluten-free bread using a mixture of both konjac and xanthan gum in a dairy-based gluten-free formulation. Furthermore research by Tye (1991) showed that konjac flour and xanthan gum interact to form a gel with unique viscoelastic properties. Xanthan gum has been used as a thickener, or mixed with other gel-forming polysaccharides. According to Christiansson and others (1974) and Christiansson (1976), to obtain a good crumb structure in the absence of gluten, xanthan, a hydrophilic polysaccharide of microbiological origin, was added to a starch-based bread. In the preparation of gluten-free bread, xanthan has been found to confer a crumb structure similar to that of bread made from normal wheat flour. It is suggested by Van Vliet and others (1992) that xanthan may act by improving the strain hardening properties of the dough. These authors suggest that a material shows strain hardening if the resistance to deformation is higher for relatively more extended test pieces. Hydroxypropylmethylcellulose (HPMC) is a popular hydrocolloid for gluten replacement in gluten-free bread systems. One of the properties of HPMC is revealed on exposure to water or biological fluid; the dry polymer becomes hydrated, swells, and forms a gel barrier layer (Kavanagh and Corrigan 2004).

45.3.8 Protein Sources in Gluten-Free Breads

The replacement of gluten with other protein sources such as soya and dairy proteins is another approach used in the production of gluten-free products. Several studies have been reported, where the inclusion of dairy proteins in gluten-free systems plays an important role. Dairy proteins, for instance, have functional properties similar to gluten. They are capable of forming networks and they have good swelling properties. Dairy proteins are highly functional ingredients and, due to their versatility, can be readily use in many food products (Gallagher and others 2003). They may be used in bakery products for both nutritional and functional benefits, including flavor and texture enhancement, and storage improvement (Cocup and Sanderson 1987; Mannie and Asp 1999; Kenny and others 2001; Gallagher and others 2003). They contribute to a number of critical characteristics for food products. These include the emulsifying and stabilizing ability of caseinates, the gelling properties of whey protein concentrates and isolates, the water-absorption capacity of high-heat nonfat dry milk, and the browning of lactose during heat processing (Chandan 1997). Furthermore, they may be used in gluten-free bread formulas (Fig. 45.4) to increase water absorption and therefore enhance the handling properties of the batter (Gallagher and others 2003). Gallagher and others (2003) found that dairy powders used in gluten-free bread formulations resulted in improved volume, appearance, and sensory aspects. However, the supplementation of gluten-free breads with high-lactose-content powders is not suitable for persons with celiac disease who have significant damage to their intestinal villi. They may not be able to utilize the lactose due to the absence of the lactase enzyme, which is normally generated by the villi.

Surimi, soyabean, and egg proteins are alternative protein sources used in baking applications. Surimi is a concentrate of myofibrillar proteins obtained after mixing and water washing of fish flesh (Han-Ching and Leinot 1993). Gormley and others (2003) focused on the supplementation of a control gluten-free bread based on rice flour and potato starch with fish surimi (as a structure enhancer and protein replacer) at a 10% inclusion level (of starch weight), and found that the addition of surimi improved the overall quality of the bread. Furthermore, regarding sensory analysis, no significant negative effects were found with the addition of the surimi to the bread.

Soya has several functional properties. Soya isoflavones (plant components of soya) have been shown to have positive effects on bone tissue in controlled *in vitro* experiments on bone cell lines and animal studies and, as well as the first results from human studies, point to a promising future for soya isoflavones for use in functional foods that target a reduction in the risks of osteoporosis (Brouns 2002). Furthermore, soya isoflavones are reported to reduce the risk of cardiovascular disease, reduce the oxidation of low-density lipoproteins (LDL), and have a role in preveting osteporosis and breast cancer. Finally soya isoflavones possess antioxidant properties. Soya flour is widely used in the production of gluten-free products. Sanchez and others (2002) reported that the inclusion of 0.5% soya to optimized gluten-free bread enhanced the crumb grain score, bread, volume, and overall bread score. Research by Jonagh and others (1968) showed that proteins such as egg albumen and gliadin at low concentrations were able to link starch granules together. According to Moore and others (2004a), egg proteins, part of the soya protein, or colloidal solutions such as caseins swell and form viscous solutions in a gluten-free bread system. Confocal laser-scanning microscopy revealed filmlike continuous protein structures similar to wheat gluten (Fig. 45.4).

45.3.9 Starch Sources in Gluten-Free Bread

In cereal-based products, starch gelatinization, as well as subsequent reorganization, controls texture and stability of the final product. Starches such as wheat starch (less than 200 ppm), rice, potato, and cornstarch are most commonly used in the production of gluten-free products. The suitability of rice flour is attributed to its low levels of prolamins (Gujral and Rossel 2004). Optimal rice bread formulations have been developed using carboxymethylcellulose and hydroxypropylmethylcellulose, which meet wheat bread reference standards for specific volume, crumb and crust color, firmness, and moisture (Collar and others 1999; Gallagher and others 2002). Rice flours can be used in baking applications in many different forms. The addition of whole-grain rice to gluten-free breads improves the quality of the bread and subsequently enhances the nutritional value. Potato starch has desirable characteristics, which differ significantly from those of starch from other plant sources (Madsen and Christensen 1996). Its functional characteristics include high swelling power, water-binding capacity, and freeze–thaw stability. Ács and others (1996a,b) used binding agents (xanthan, guar gum, locust bean gum, and tragant) as a substitute for gluten in a gluten-free bread formulation based on cornstarch. These authors concluded that the binding agents resulted in an increase in loaf volume and loosening of the crumb structure.

45.4 CONCLUSION

Celiac disease, which is an autoimmune enthalpy characterized by the inflammation of the small-intestinal mucosa, is more common than originally estimated. According to epidemiological studies, approximately 1 in 80 of the world population is suffering from this disorder. This segment of the population has been deprived of good quality cereal products, which is part of the staple diet for the majority of human beings. One of the biggest problems for the food scientist will be to provide the consumer with good quality products. Gluten has unique properties in relation to production of good quality cereal products, which make its replacement so very difficult. From the evidence gathered, it becomes apparent that a mixture of different flour, gums, and proteins is necessary to replace gluten and produce good quality gluten-free cereal products. The development of gluten-free cereal products is an opportunity to diversify cereal products as a market, keeping in mind the need to provide the consumer with products of high nutritional value.

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Volume 1 Index

2-Acetylthiazole, 458
2-Alkanols, 186
2-Alkanone, 186
α-Acetohydroxy acids, 477–478, 488
α-Acetohydroxybutyrate, 488
α-Acetolactate decarboxylase, 478
α-Acetolactate, 477, 479, 483, 485, 488, 490
α-Amylase, 449–451, 457
β-Amylase, 447, 449–451
AAD gene, 473
Acesulfame K, 310
Acetaldehyde, 182, 186, 471, 479, 481, 851–859
Acetate, 475
Acetic acid, 729–730, 735
Acetoin, 471, 478, 717
Acetone, 212
Acid (also acidity, also titratable acidity), 568, 569, 572, 573, 574, 576, 578, 579
Acid food(s), 225, 235
Acid-coagulated cheeses, 636
Acidification. *See* Fermentation
Acidophilus milk, 62, 63
Aconitase, 476
Active amyl alcohol, 471, 473
Acyl-coenzyme A, 474
Additives, 609
Adenylylsulphate kinase, 481
Adjuncts, 447, 457, 468, 478
Adjunct culture, 572–573, 578
AEDA. *See* Aroma extract dilution analysis
Aeration, 588
Aflatoxin contamination, 698
Aftertaste, 288, 293
Agaricus bisporus, 183
Aggregation, 578, 653, 658
Aging, 584
AGT1 gene, 470
Alcohol acyltransferase, 474
Alcoholic beverages, 181–182
Alcohol dehydrogenase, 472–473, 478
Alcoholic fermentation, 181
Alcohols, 181, 186, 210, 849
Aldehydes, 181, 186, 210
Alitame, 310
Allergen(s), 284, 299
Alliin, 916
Allyl isothiocyanate, 125
Alpha-lactalbumin. *See* Whey protein
Alpha-linolenic acd (ALA), 915
Alternative (new) technologies, 38–42
Alternative sweeteners, 310
  acesulfame K, 310
  alitame, 310

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

Alternative sweeteners (*Continued*)
aspartame, 310
neotame, 310
sucralose, 310
Alzheimer's disease (AD), 914, 920
Amaranth, 1023–1024
American Bottlers of Carbonated Beverages (ABCB), 421
American Heart Association, 919
American Spice Trade Association, 214
American whiskey, 517
Bourbon, 517
charring, 518
corn whiskey, 518
distillation, 518
column still (patent still), 518
Kentucky bourbon, 518
maple charcoal, 518
neutral grain spirit, 518
rye whiskey, 518
sour mash, 517
backset (setback), 517
sweet mash, 518
Tennessee whiskey, 518
American-type Camembert cheese manufacturing, 56, 58
AMF mixer, 321
Amino acids
anabolic pathway, 472–473
catabolic (Ehrlich) pathway, 472–473
isoleucine, 472–474, 477
leucine, 472–474, 477
methionine, 478, 480–481
phenylalanine, 473
threonine, 478, 484
tryptophan, 473
valine, 472–474, 477
Amino acids metabolism, 472, 475, 477
Aminotransferase, 1027
Amount of alcohol in candy, 699
Amyl alcohol, 182
Amylases, 188
Anethole, 210
Angel food cake, 317
Animal and marine fats and oils, 107–108
Anise, 210, 213
Annatto. *See* Color
Anthriscus sylvestris, 167
Antimicrobials, 885–886
carbonyl compounds, 885
essential oils, 885–886
organic acids, 885
Antimycotic agents, 573
Antioxidants, 129, 209, 993, 998, 1005
Apple cider, 856
Apple juice, 848–860
Apple: production, chemistry and processing, 773–791
apple varieties, 777
composition, 780
consumption, 775
harvest and storage, 776
leading country, 774
leading fruits, 774
organic apples, 774
physico-chemical characteristics, 779–783
apple flavor, 779
nutrional quality, 782
phenolic compounds and antioxidant capacity, 780
post harvest handling, 776–777
processed apple products, 783–789
apple juice and cider, 784–786
apple slices, 786
applesauce, 787
dried apples, 787
freeze-dried, 789
glass transition, 787
infused dried, 789
processed frozen apples, 787
water activity, 789
supply, 774
Apples, 841–854
Arginine, 977, 1004
Aroma, 279, 293
Aromatic herbs, 209
Aromatic profiles, 141
Ascomycetes, 183
Aseptic packaging, 858–859
Ash level, 216
Asian (Oriental) noodles
classification, 541
history, 539–562
variables affecting quality, 561–562
Aspartame, 310
Aspergillus, 188
Aspergillus niger, 186–188, 884
Aspergillus parasiticus, 187
ASTA. *See* American Spice Trade Association
ASTA color value, 217
ATF1 gene, 474–475
ATF2 gene, 474–475
Aureobasidium, 186

Authenticity, 215
Available water, 216
Azeotropic distillation, 159

2,3-Butanedione, 477
Bacillus subtilis, 187
Bacteriophage, 487, 572, 574, 588
Bacteriostatic, 602–603
Bagel(s), 293–299
  evaluation, 299
  New York style, 295
  troubleshooting, 299
Baker Perkins homogenizer, 321
Baker's percent, 285, 286, 294, 300
Bakery, 262–266, 271–274
  biscuit, 262, 264, 266–267, 269, 271, 273
    checking, 264
    cracking, 264
  bread, 262–263, 265, 268–269, 271–274
    sourdough, 263
    whole grain, 262
    whole wheat, 262
  cake, 263–264, 271–272
    batter, 263
    emulsion, 263–264
  pastry, 265
Bakery products, 102–103, 183
Baking, 291, 297, 315–316, 321
Baking powder, 285, 289, 312
  double acting, 289, 312
  single acting, 312
Baking soda, 285, 312
Banana, 817–826, 867
  classification, 817
    Cavendish, 817
    Grand Nain, 817
    Gros Michel, 817
    Lacatan, 817
    Lady Finger, 817
    Musa acuminata, 817
    Señorita, 817
  cultivation, 817
    finger, 817–818
    hand, 817–818
    inflorescence, 817–818
    suckers, 817–818
    tissue culture, 817
  figs, 868, 872–873
  fingers, 819, 872
  flour, 868, 873
  harvest, 819–820
    inflorescence, 819
    latex, 819
    tramline, 819
  nutritional quality, 820
    potassium, 820
    starch, 820
    sugars, 820
  nutritional value, 837, 873
  phenolics and antioxidant capacity, 821
    dopamine, 820
  post-harvest handling, 819–820
    chilling injury, 820
    color index scale, 820
    ethylene ripening, 819
    ripening, 820
  powder, 873
  production, 818–819
    yield, 819
  products and processing, 821–826
    chips, 821, 825
    individually quick frozen, 825–826
    osmotic dehydration, 825, 873, 875–876
    other banana products, 821–826
    puree, 821–825
  storage, 820
  supply, 818
Band ovens, 323
Basidiomycetes, 183
Batter(s), 225, 234, 291, 319–321
  depositing, 291, 321
Beads
  calcium alginate, 487
  calcium pectate, 487
  ceramic, 483, 487, 489
  DEAE-cellulose, 487–490
  porous glass, 487–488
Beer, 174, 182, 195
  alcohol-free, 487–489
  bitterness, 456–457, 479
  body, 451, 479
  brewing process, 404, 444–446, 455, 485, 487
  color, 450, 457–458, 466, 479
  filtration, 447–449, 451, 455, 467
  green, 446, 478, 481, 485, 488, 490
  history, 444
  low-alcohol, 488
  packaging, 446
  stabilization, 446, 483
Beef extract replacer, 133
Beef, 921

Beet sugar, 104
Beiju, 186
Bell pepper, 219
Beneficial microflora, 985
Benzaldehyde, 202
Benzoic acid, 917
Benzyl alcohol, 852
Beta-lactoglobuline. *See* Whey protein
Beverage technologists, 429
Beverages, 108–111
Bifidobacteria, 181
  compounds, 998, 1000, 1002, 1004, 1006–1010
  peptides, 991, 1007
Bioavailability, 993–994, 1008–1010
Biogenic amines, 1007–1008
Bioprocess, 170–171
Bioreactors, 198–200
  packed-bed, 485, 487–488
Biosynthesis, 180
Bioterrorism Act of 2002, 246
Biotransformation, 180
Bis-(2-methyl-3-furyl)-disulfide, 859
Bitter, 611, 628
Bjerkandera adusta, 202
Black pepper, 212
Blanching, 225
Blend, 597, 605–606
Blue cheese, 600
Blue cheese manufacturing, 55
Blueberries, 805–812
  average production, 805
  blueberry products, 811
    blueberry juice and concentrate, 811
    blueberry puree, 811
    infused and stabilized blueberries, 811
    Infused-dried, 811
    IQF blueberries, 811
  characteristics of selected varieties, 781
  demand, 807
  physico-chemical, phytonutrients, and nutritional qualities, 796, 808
    anthocyanins, phenolics and antioxidant capacity, 808
    chemopreventive and anti-adhesion properties, 810
    effect of processing on phytonutrient quality, 809
    flavor compounds, 808
    nutrients in blueberries, 810
Boiling, 297
Bone health, 923
Bottled and canned soft drinks and carbonated waters, 110
Bound moisture, 163
Bovine spongiform encephalopathy, 133
Bread, 12, 66–70, 354
  Altamura bread, *pane di Altamura*, 367
  basic steps, 68
  bread substitutes, *grissini* and *taralli*, 368
  characteristics, 355
  functional ingredients, 67
  process, 359
  raw materials, 354, 356
  recipes, 69
  shelf life, 356, 358
  types, 66
  various breadmaking processes, 68
Bread and other bakery products except cookies and crackers, 102–103
Bread-making, 359
  sourdough, 361
  sponge and dough method, 361
  straight dough method, 360, 363
Brevibacterium linens, 181
Brie, 181
Brine cured ham manufacturing, 65
Brix/acid ratio, 852
Brown sugar, 310
Browning, 9, 26, 27
  ascorbic acid, 9, 33
  citric acid, 34
  cysteine, 886–887
  enzymatic browning, 786, 821, 825, 887, 889
  phenolic substrates, 883
  polyphenol oxidase, 821, 886
  quinones, 883, 886
  sodium erythorbate, 887
Browning Maillard, 301, 310, 318
Buckwheat, 376–377
  composition, 379
  gluten-free bread, 381
  pizzoccheri, 378
  typical products, 369
Buffering, 602–603
Bulk density, 216
Bulking agents, 311
Bun tau, 559
Butanoic acid, 981
Butanol, 185
Butter, 705, 717–718, 721–722

Butter clarified, 318
Butter drawn, 318
Butterfat, 653–655
Buttermilk, 728
Butyl acetate, 853–854
Butyric acetate, 184
Butyric acid, 182, 732

α_{s1}-Casein, 975
α_{s2}-Casein, 973, 975
β-Caryophyllene, 210, 853
β-Casein, 741, 973
κ-Casein, 973–974
Cadin-4-ene-1-ol, 187
Cajun dishes, 211
Cake flour, 308
Cake mixing, 320
Cakes, 308, 314, 317
  foam-style, 312, 317
  shortened-style, 314, 316
Calcium, 602–603, 607–608, 619, 652, 654, 729, 743
  calcium chloride, 570–571, 576
  calcium lactate crystals, 589
  calcium phosphate, 570, 578, 581, 586, 589
Calories, 991–992, 1001
Camembert, 56, 181
Canadian whisky, 518
  base whisky, 518
  flavoring whisky, 518
  rye whisky, 518
Cancer, 913
Candida, 181, 183, 187
Candida cylindracea, 186
Candida utilis, 183, 186
Candy and other confectionery products, 104–105
  deceptive products, 699
  equipment and manufacturing processes, 698
  raw materials, 696
  sample collection, 699
Cane sugar except refining, 103–104
Cane sugar refining, 104
Canned and cured fish and seafood, 111
Canned fruits, vegetables, preserves, jams, and jellies, 95–96
Canned tuna, 19
Canned, frozen, and preserved fruits, vegetables, 94
Canning, 857
Capric acid, 736
Capsaicin, 212, 217
Capsicum, 217
Caraway, 210
Carbohydrates, 1001, 1004
  fermentable carbohydrates, 920
Carbon dioxide (gas carbonium), 420
  analytical procedure for bottler's carbon dioxide, 433
  feed gas sources, 433
  purification, 433
  quality guidelines for bottler's carbon dioxide, 433
  quality verification levels, 433
  safety, 434
  storage, 434
Carbon dioxide, 446, 471, 479, 482
Carbonated soft drinks (CSDs, soft drinks, soda pops)
  facts and figures, 421
  history, 420
  process of manufacturing, 422
    concentrates, 533–534
    filling, sealing, and packing, 437
    finished product, 440–441
    overall process, 271, 422
    quality control and assurance, 439–440
    sweeteners, 423, 429
    syrup preparation, 434
    water, 425
Carbonation, 421–422, 436–437
  Bunsen coefficient, 422
  carbonating technology, 437
  control, 436
  favorable results, 421
  gas volume, 422
  process, 421
  reactions, 421
Carbonyls, 186
Carboxylic acids, 210
Cardamom, 167, 213
Carotenoids, 934, 998, 1005
Carrier materials, 486–487
  selection criteria, 486
Carrot, 219
Carveol, 187
Carvone, 210
Caryophyllene, 858
Case hardening, 869
Casein, 579, 581–582, 584, 589–590
Casein micelles, 648, 652, 974
Casein, functional peptides derived from, 974

Cassava, 186
Catabolite inactivation, 469
Catechins, 918
Cat's ear noodles, 544
CCP, 238
Celiac disease, 1018–1020, 1022
  clinical symptoms, 1019
  diagnostic procedures, 1018
  epidemiology, 1019
  iceberg model, 1019
  possible causes, 1019
Cell, 289, 291
  size, 289, 293
  uniformity, 293
  walls, 293
Cellophane noodles, 559
Cellulases, 188
Centrifugation, 162
Ceratocystis, 185
Ceratocystis fimbriata, 194, 196–197, 200, 202
Ceratocystis moniliformis, 187, 202
Ceratocystis variospora, 187
Cereal breakfast foods, 99
CFR, 225
CGMPR, 224, 243
Cheddar, 582–590
Cheddar cheese manufacturing, 54, 56
Cheeses, 48–51, 652
  approximate weight, 53
  basic cheese-making steps, 54
  classifications, 52
  packaging material requirements, 53
Cheese types
  American cheese, 596, 600
  Colby, 569, 572–573, 575, 582, 586
  cold-pack cheese food, 596–597, 599
  cold-pack cheese spread, 600
  cottage, 598–599, 618–619
  Jack. *See* Monterey
  Longhorn, 569
  Monterey, 569–570, 582, 584
  pasteurized process cheese, 596–603, 606, 608, 610, 612, 614
  pasteurized process cheese product, 599–600, 611
  pasteurized process cheese spread, 597, 599, 606
Chemical composition of milk, 972
Chervil, 209
Chewing gum, 106
Chhana, 638–639
  manufacture, 639
Chiffon cake, 318
Chili, 219
Chili pepper, 212
Chilling and refrigeration processes, 27–28
Chinese Jinghua ham processing, 64
Chinese pickled vegetables, 79–80
  Basic steps, 80
Chitin, 891
Chives, 209
Chlorination, flour, 309
Chocolate, 919
  bloom, 690
  conching, 683
  depositing, 687
  enrobing, 686
  liquor, 676
  manufacturing, 682–686
  molding, 687–688
  nutrition, 691–693
  rancidity, 691
  refining, 682–683
  storage, 691
  sugar-free, 689–690
  tempering, 684–686
  U.S. standards, 680–681
  viscosity, 683–684
Chocolate and cocoa products establishment inspection, 696–698
  processing, 697
  raw materials, 696–697
  sample collection, 697–698
Chocolate and cocoa products, 105–106
Chocolate coating
  compound, 688–689
  sugar free, 689–690
Chocolate, 919
Cholesterol, 189, 719
Chymosin (also rennet), 572, 578
Cinnamaldehyde, 210
Cinnamic aldehyde, 211
Cinnamon, 211, 213–214
CIP, 241
Cis-3-hexenal, 857
Cis-3-hexenol, 858
Citral, 187, 853, 858
Citrate, 476, 728
Citric acid, 604, 853
Citronella, 167
Citronellal, 187

Citronellol, 187
Citrus
  flavonoids, 918–919
  fruits, 917
  limonoids, 917
Citrus juice, 845–860
Cleaning, 21
Cleanliness, 215–216, 226
Clevenger apparatus, 149
Clinical nutrition products, 969
Clostridium butyricum, 197
Clove, 213
Clumping. *See* Partial coalescence
Coagulation, 286, 456–457, 463
Coalescence, 658
Cocoa
  powder, 678–682
Cocoa beans, 696
  filth, 696
  fumigation, 696
  insect infestation, 696
  inspection, 696
  microbial tetsing, 696
  moldy beans, 696
  preliminary field examination, 696
  Salmonella Free Certificate, 697
Codex Alimentarious, 236–237, 283, 1021
Codex Alimentarius Commission, 136
Codex classification, 207
Cold liquid freezers, 31
Cold press, 211
Cold pressing, 152
Cold-trap on-line sampling method, 396–400
Colon cancer, 920
Colloidal stability, 451, 457, 466, 479
Color Additives Status List (CASL), 253
Color additives, 35
Color values, 217
Color, 569, 573, 576–577
Colostrum, 739
Composite, 656
Composition
  cow's milk, 47, 972
  wheat, flour, and germ, 67
Conching of chocolate, 683
Conduction heat, 322
Conjugated linoleic acid (CLA), 921
Consumer Health Information for Better Nutrition Initiative of the U.S. Government, 929
Consumer use, 665
Consumption, 596, 611
Continuous mixers, 320
Continuous subcritical water extraction, 152, 154
Control of foreign objects, 698
Controlled atmospheric storage (CAS), 28
Convection heat, 322
Convection oven, 322
Conventional method, 314
Conventional meringue method, 315
Cookies and crackers, 103
Cooking, 579–581, 585, 598, 607, 622
Cooling, 261, 266, 270, 274
  air blast cooler, 270
  natural cooling, 270
  vacuum cooling, 270
Co-PET, 860
Corn, 370
  dry milling, 373
  gluten-free pasta, 376
  milling fractions, 373
  polenta, 374
  wet milling, 372
Cottage cheese manufacturing, 54–55
Cottonseed oil mills, 106
Crabtree effect, 471
Cranberries, 917
  nondialysable polymeric compound, 917
Cream, 652
  age gelation, 743
  agglutinin reaction, 735, 739
  air cells, 734, 741–742
  churning, 742
  composition, 726–727
  creaming, 735
  feathering, 743
  frozen, 738
  Kreiger–Dougherty equation, 736
  lipolysis, 732–733
  off-aroma, removal of, 741
  Ostwald ripening, 741
  over-run, 740–741
  pasteurization, 727–728, 733, 740
  re-bodying effect, 736, 738–739
  recombined, 737
  rheology, 683, 687
  separation, 728
  spinning cone technology, 745
  stabilizers, 737, 741, 743
  Stokes equation, 735
  vacreation, 745
  volatiles, 745

Cream cheese, 652–667
Cream dressing (also dressing, also creaming), 618, 620–621, 625, 627–628
Cream of Tartar, 313, 317
Cream, types of
  clotted, 739
  double, 739
  dried, 740
  half and half, 739, 742
  heavy, 739
  light, 739
  liqueur, 744
  plastic, 736
  sterilized, 742
  ultra-high temperature (UHT) treated, 733
  whipping, 740–742
Creamery butter, 91
Creaming, 312
Creaming method, 314
Critical Control Point (CCP), 225, 236–239, 242
Cryogenic cooling, 27
Croissant, 70
Cruciferous vegetables, 916–917
Crumb, 300
Crust, 287, 292
  blisters, 298
  color, 288, 292, 298
Cryogenic freezers, 31
Cryoglobulins, 738–739
CSWE. *See* Continuous subcritical water extraction
Cu mien (thick noodles), 543
Culinary herbs, 122
Culture, 569–570, 572, 574, 576, 578, 587–588, 619, 622
Cultures
  probiotic, 985
  yogurt, 984
Cumin, 213
Curcumene, 210
Curcumin, 217
Current Good Manufacturing Practice Regulations (CGMPR), 224
Cutting, 579–580
Cymbopogon winterianus, 167

2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 855
2,5-Dimethylpyrazine, 392, 402
4-Decalactone, 187, 196
β-Damascenone, 855, 861
γ-Decalactone, 172, 201
®-δ-Dodecanolide, 196
Dai mien, 544
Dairy products, 920
Dang myun, 561
Danish pastries, 70–71
D-carvone, 210
Debaromyces, 181
Decanal, 857
Decoction, 447, 451–452, 455, 458
Depositing batter, 321
  types, 321
Derived flavor, 121
Dextrins, 450, 468
Diacetyl, 175, 182, 471, 477–481, 485, 488–490
Dietary fiber, 1004, 1015, 1020
  Inulin, 1023
Dietary nutrient
  goals, 991
  intake, 991
Dietary supplements, 940
  Canada, 941
  Europe, 941
  Japan, 941
  United States, 941
Diglycerides, 212, 710
Dilactone, 853
Dill oil, 210
Dimethylanthramilate, 853
Direct dryer, 164
Directly acidified cheeses, 648
Disease, 990–991, 997, 1001
Disintegrating, 22
Disodium 5′-guanylate, 123, 129
Disodium 5′-inosinate, 123, 129
Distillation, 149, 157–162
Distillation columns, 158
Distilled and blended liquors, 109–110
D-limonene, 860
DMHF. *See* 4-Hydroxy-2, 5-dimethyl-3(2H)-furanone
Dog and cat food, 101
Double acting baking powder, 312
Douchi, 73
Dough making, 265, 267–268, 274
  energy input, 267
  flour hydration, 267
  mixing, 263, 266–267, 271–273
  rheological characteristics, 267

water addition, 265–266
Dr Brian Lawrence's bible, 211
Draining, 576, 582
Dried and dehydrated fruits, vegetables, and soup mixes, 96
Drier, 870
convective air, 870
tunnel, 870
Dry cured ham manufacturing, 63–64
Dry, condensed, and evaporated dairy products, 92–93
Drying, 162–167, 867–869
Drying effects, 166–167
freeze-, 872
microwave, 871
solar, 869
sun, 869
D-tagatose, 923

(−) Epicatechin, 919
Echinacea, 925
Edible coatings, 890–891
lipid, 890
polysaccharides, 890–891
alginates, 890
casein, 890
celluloses, 890
chitosan, 890–891
pectin, 890
proteins, 890
Education and training, 227
Egg noodles, 546–549
Eggs, 289, 312–313
foam, 312
substitutes, 313
whites, 313
yolks, 313
Elettaria cardamomum, 167
Embden-Meyerhof-Parnas pathway, 181, 471
EMC. *See* Enzyme-modified cheese
E-min, 550
Emmental, 596, 605
Emulsifiers, 311
Emulsifying agents, 596, 601–604, 606–608, 613
citrate(s), 601–602, 607
phosphate(s) (mono, ortho, poly, pryo), 601–603, 607–608
trisodium citrate, 603, 607
trisodium phosphate, 602
Emulsion, 603, 605–606, 610, 655–656
Encapsulated flavorings, 125–126
Enfleurage, 148–149, 155
Enhancer, 128
Enzymatic technology, 188–189
Enzyme(s), 569–573, 575, 578, 580, 584, 587, 600–601, 613, 620, 623, 726–727, 732
Enzyme-modified cheese, 188–189
EO. *See* Essential oils
Ephedra (Ma huang, herbal ectasy, mahuanggen), 925
Ergosterol, 474, 482
Erwinia caratovora, 186
ESA. *See* European Spice Association
Essential oils, 148, 160–162, 180, 205–220
Esters, 181, 183–186, 210, 471, 473–474, 476, 488, 852
acetate, 475
biosynthesis, 476
C6-C10 medium chain fatty acid ethyl, 473
synthetase, 480
Estragole, 210
Ethanol acetyltransferase, 475
Ethanol, 444, 470, 489
Ethyl acetate, 182–186, 197, 212, 474–475, 485, 858
Ethyl acetoacetate, 392
Ethyl butanoate, 852–858
Ethyl butyrate, 183, 186, 780
Ethyl caproate, 474
Ethyl caprylate, 475
Ethyl hexanoate, 474, 853, 861
Ethyl octanoate, 474
Ethyl propionate, 185, 853–854
Ethyl *trans*-2-*cis*-4-decadienoate, 854
Ethyl 2-methylbutanoate, 848, 852–853
Ethyl-2-octenoate, 854
Ethyl-4-decenoate, 854
Ethylene oxide, 217
Ethylene vinyl alcohol co-polymer, 859–860
ETO. *See* Ethylene oxide
Eugenol, 210
European Spice Association, 214
Evaluation (also defects) 584, 628
Evaporation and drying, 31–32
Evaporative cooling, 27, 410–411
EVOH. *See* Ethylene vinyl alcohol co-polymer

Exclusion chromatography, 153
Extraction modes, 147–155
Extraction using microwaves, 153
Extraction with hot fat, 149, 155
Extraction with organic solvents, 149–150
Extraction with sonic waves, 153
Extraction with supercritical carbon dioxide, 150–152
Extractive distillation, 154, 159
Exudation. *See* Syneresis

FAO/WHO, 283
Fat, 287, 296, 596, 598, 604, 618, 659, 990–991, 993, 996–1001, 1003–1004, 1008. *See also* Lipids
  emulsified, 311
  fatness, 996
  hydrogenated, 302
  low, 993, 997–998, 1000, 1006
  oil, 296, 311
    shortening, 296, 311
  reduction, 990–991, 993, 995, 998, 1000
  replacements, 1001, 1004
  substitutes, 287, 1001
  trans, 302
Fat globules
  size, 727, 730–731
  stability, 729–730, 733–735
  surface load, 729
  surface tension, 730, 733, 738
  zeta potential, 727
Fat replacer(s), 288, 300
  carbohydrate based, 288
  lipid based, 281
Fats and oils, 106–108
Fatty acids, 467, 471, 474, 482
  unsaturated, 474, 482
FDA, 224, 233, 240, 300
  Food Code, 224, 240, 243
Fen si, 559
Fennel, 213
Fennel oils, 210
Fermentation, 38, 180–181, 206, 663
  continuous, 426, 428
  high-gravity, 468–469, 474, 483
  main/primary, 457, 478
  secondary, 479, 482, 485, 488
Fermentation process types, 196–200
Fermentation systems, 194–200
Fermented cereal products, 66–71
  ingredients, 66
  types, 66
Fermented dairy products, 46–63
  common organisms used, 50
  dairy starter cultures, 51
  ingredients, 46, 49
  kinds, 46
  temperature requirements, 52
  types and examples, 48
Fermented foods
  application of biotechnology in manufacturing, 80
  process mechanization, 80–81
Fermented liquid milks, 59–63
Fermented meat products, 63
  ingredients, 63–64
  types, 63
Fermented soy pastes, 74–75
Fermented soy products, 71–77
  ingredients, 71–72
  types, 71
Fermented tofu, 75–76
Fermented vegetables, 77–80
  kinds, 79
  raw ingredients, 77
Fermented whole soybeans, 73–74
Ferulic acid, 861
Feta cheese manufacturing, 57, 59
Feverfew, 925
FFA. *See* Free fatty acids
Fiber, 982
Filling, 665
Finished product sampling, 697–698
Fish, 919
Flammulina velutipes, 183
Flat (layered) bread, 69–70
Flavonoids, 915, 917
Flavor, 169–174, 207, 293, 569–575, 581, 583–585, 601, 603, 605, 611
  cardboard, 481
  fullness, 451, 479, 481
  stability, 451, 457, 479
  threshold, 473, 476, 477, 480
Flavor chemists, 139
Flavor compounds
  biosynthesis, 183–187
  extraction, 200–202
  physical and chemical properties, 390
  produced by fungi, yeasts, and bacteria, 179–189
  thermal stability, 392–393
Flavor concentration
  on-line measurement apparatus, 391–392
Flavor extraction, 148–154

Flavor from fermented foods, 180–187
Flavor integers, 127
Flavor migration
in solid food matrices, 390–413
Flavor migration
system development, 391–393
Flavor migration, 390–413
Flavor migration in formulated dough, 400–408
Flavor perception, 127–129
Flavor production, 194
effect of environmental conditions, 194
Flavor profile, 139
Flavor profile analysis, 143
Flavoring extracts and flavoring syrups, not elsewhere classified, 110–111
Flavorist, 129, 139–140
Flaxseed, 915
Flocculation, 483–484
Flour and other grain mill products, 98
Flour, 265, 286, 295, 308, 309
all purpose flour, 286
cake flour, 308
self-rising flour, 309
whole wheat, 286
Flour, common wheat, 332, 344, 355
rheological characteristics, 332
Fluid milk, 93–94
Foam stability, 458, 463, 466, 479
Foam style cakes, 317
angel food cake, 317
chiffon cake, 318
Genoise cake, 318
sponge cake, yellow, 318
Foaming, 438
Folding, 312
Food additives, 36
Adverse Reaction Monitoring System (ARMS), 36
approval process, 906
common uses in food categories, 34
Delaney clause, 36
direct food additives, 34
Generally Recognized As Safe (GRAS), 35
indirect food additives, 34
regulations, 35–36
why, 33
Food Additives Amendment, 252
Food Allergen Labeling and Consumer Protection Act of 2004, 284
Food and Agricultural Organization (FAO), 11
Food and Agriculture Organization of the United Nations, 283
World Health Organization (FAO/WHO), 283
Food and color additives, 698–699
Food and Drug Administration (FDA), 246
Food and drugs act, 135
Food Code, 224–242
Food handling and processing, 10
Food labeling, 283
Food laws, 136
Food manufacturing (procesing) goals, 6
Food manufacturing plant
construction, design and maintenance, 248
environment, 247
plant services, 248
waste disposal, 248
Food odor research, 140
Food packaging and storage, 10
Food plant inspection, 246–252
equipment and utensils, 249–250
manufacturing process, 251–255
color additives, 253
food additives, 252
formulas, 251–252
ingredient handling, 251
packaging and labelling, 254–255
quality control, 253
personnel, 246–247
plants and grounds, 247–248
preparation, 246
raw materials, 249
handing procedures, 249
Food preparations, not elsewhere classified, 113–114
Food preservation, 10
Food spoilage
biological factors, 7
chemical (including biochemical) factors, 8
physical factors, 9
prevention and retardation, 10
sources of information, 11
Food standards, 252
Food Standards Agency of the United Kingdom, 283
Food Standards Australia, New Zealand, 283
Food technologist, 11
Foodborne disease
common bacteria genera, 8
most common pathogenic bacteria and viruses, 8
Foodborne infection, 8

Foodborne intoxication, 8
Foods For Specified Health Use (FOSHU), 906
Forming, 23
  confectionery, 23
  meat and poultry patties, 23
  pasta, 23
Formula, 288
Formulation, 143
Fortification, 968
Fractional distillation, 141
Fractionator, 159
Frankfurters, 12
Free fatty acids, 9, 689
Free moisture, 163
Free radicals, 858
Freeze-drying, 166
Freezing and frozen storage, 29
  principles, 29
  quality changes, 29
  rate of freezing, 29
French paradox, 918
Fresh herbs, 209
Fresh pasta, 339
  classification criteria, 340
  cooking quality, 333
  kneading and sheeting, 345
  packaging, 350
  pasteurization, 349
  quality characteristics, 331, 335, 367
  raw materials, 343
  shelf-life, 352
  stuffed pasta, 341
  water activity, 196
Fromage frais, 652
Frozen bakery products, except bread, 103
Frozen chicken parts, 12
Frozen fruits, fruit juices, and vegetables, 97
Frozen specialities, not elsewhere classified, 97
Fructose, 468, 689
Fruit juices, 855
  packaging, 857–859
  storage, 861
Fruit juice processing, 855
FUM1 gene, 476
Fumarase, 476, 489
Fumarate reductase, 476
Functional food(s), 904–906, 913–928, 1000–1004, 1007, 1023
  biological effects, 914–922
  consumers' focus on prevention of illness, 947
  definition, 906
  dietary guidance, 929–930
  effects on major chronic diseases, 913–914
  examples, 906
  history of use, 945
  meat-based
    animal production practises, 995, 1003
    meat transformation systems, 999
  public education, 929–930
  regulation of, 927–929
  safety issues, 924–925
  safety and toxicology aspects related to functional, 945
  the global market, 942, 953
  the rising cost of health-care related to chronic disease, 943
  the role of food in prevention strategies, 945
Functional foods ingredients, 904
Functional foods, nutraceuticals, 958
  Europe, 941
  Japan, 941
  United States, 941
Functional ingredients, 959, 961
Functional properties of milk, 971
  enhancement by culturing, 984
Functional role of calcium and other minerals, 982
Functionality, 311
Fungi, 33
Furaneol, 798, 836, 855
Furanones, 798
Furans, 850
Furfural, 517, 828
Fusel alcohols, 181–182, 471, 475

α-Glucosidase, 188, 468, 481
β-Glucosidase, 188
β-Glucan, 445, 449–451
Garlic, 79, 121–122, 127
Gas chromatography, 210, 392–400, 845
Gas chromatography–mass spectrometry, 141, 210, 759
Gas laws of Henry, Boyle, and Charles, 422
Gas-solid fluidized-bed, 199
GC. *See* Gas chromatography
GC-MS. *See* Gas chromatography-mass spectrometry
GDP1 gene, 471
Gelatinization, 291, 308
Generally Recognized As Safe (GRAS), 35

Genetic engineering, 171–174
Genetically modified organism (GMO), 194, 301
Genoise cake, 318
Geosmin, 187
Geotrichum penicilliatum, 185
Geranial, 852
Geranic acid, 187
Geranyl acetate, 853
Ginger, 926
Gingko biloba, 926
Glass transition temperature, 406, 872
Glucose, 467–469, 689, 850
Glucosinolates, 913
Glutamic acid, 183, 994
Glutathione, 886, 977
Gluten, 100, 263, 265, 284
  replacers, 531
  vital wheat gluten, 294–295, 299, 1000
Gluten-free
  bread(s), 1021–1023
  cereals, 1021
  diets, 1019, 1021
  foods, 1024
Gluten-free products, 376, 531
Gluten proteins
  gliadin, 265, 1018, 1021
  glutenin, 265
  prolamin, 1081, 1021
Glycerol, 710
Glycerol-3-phosphate dehydrogenase, 471
Glycolysis, 471
GM, 284
GMP, 224
GMPs. *See* Good manufacturing procedures
Goat, 569, 739
Good manufacturing practices (GMP), 284
Good manufacturing procedures, 217
Gouda, 188
Grain mill products, 98–102
Granulated sugar, 314
Grape juice, 848, 855
Grapefruit juice, 848–853
Grapes, 855
Gruyere, 598
Guaco, 166
Guide to Nutritional Labeling and Education Act (NLEA) requirements, 255
Gums, 60, 599, 966

1-Hexanol, 860
3-Hexen-1-ol, 856
3-Hexenal, 856
4-Hydroxy-2,5-dimethyl-3(2H)-furanone, 861
4-Hydroxydecanoic acid, 197
5-Hydroxymethylfurfural, 458
α-Hydroxyglutarate, 476
H_2S, 480, 717
HACCP (Hazard Analysis Critical control points), 10, 224–241
HADH. *See* Hydroxyl acid dehydrogenase
Hama-natto, 73
Hams, 63, 65
Hand-cut noodles, 544
Hand-swung noodle, 544
Hand-washing facilities, 230
Hansenula anomala, 185
Hansenula mrakii, 185
Hard drinks, 420
Harusame, 541, 559, 561
Hayamugi, 543
HDL cholesterol, 918, 923
HDPE. *See* High-density polyethylene
Headspace vapor analysis, 140
Healing, 579, 624
Health Canada, 283
Health claims, 283
  qualified health claims, 924
    antioxidant vitamins and cancer, 924
    folic acid and neural tube birth defects, 924
    nuts and heart disease, 924
    omega-3 fatty acids and coronary heart disease, 924
    phosphatidylserine and cognitive dysfunction and dementia, 924
    selenium and cancer, 924
Health concerns, 280
Health promoting ingredients, 968
Heat application, 24
Heat blanching, 234
Heat exchangers, 25–28
Heat removal (cold preservation), 26
Heat transfer, 322
  conduction, 322
  convection, 322
  radiation, 322
Heavy metals, 216
Herbs, 206, 208, 209
  freeze-drying, 209
  in the food industry, 217–219
Herbs and spices
  quality aspects, 215–217
Herring, 111

Heterocyclic amines, 1006–1007
Hexanal, 849, 856
Hexanol, 186
Hexose transporters, 468
Hexyl acetate, 851–852
High altitude adjustment, 316, 317
High flavor impact, 121
High fructose corn syrup (HFCS), 430
  sensory active compounds, 430
High pressure processing (HPP), 40
High ratio sugar cake, 309
High vacuum isolation/aroma extract dilution analysis, 732
High-density polyethylene, 859
Higher alcohols, 181–182, 458, 471, 472, 473, 477
  aliphatic, 471
  aromatic, 471
  biosynthesis, 471
High-voltage arc discharge, 41
Hiramen, 544
Hispanic cheeses, 648
HMF. *See* 5-Hydroxymethylfurfural
Ho fen, 540
Hokkien-type noodles, 552
Homogenization, 575, 609, 621. *See also* Emulsion
  homogenizers, types of, 729–730
  lipolysis, effect on cream, 732–733
  two-stage, 729, 731–732
Honey, 310
Hydrocolloids, 604, 613, 1027
Hydroxypropylmethylcellulose (HPMC), 531, 1028
Hydrodistillation, 149, 153–155
Hydrogen sulfide, 480
Hydroxyl acid dehydrogenase, 725
Hydroxymethylfurfural, 861

β-Ionone, 187
IAH1 gene, 475
Ice, 235
Ice-cream, 733–734
Ice-cream and frozen desserts, 93
IDH1 gene, 476
IDH2 gene, 476
ILV pathway, 477, 479
Imitation flavors, 139
Immobilized cell technology, 485
  applications, 482
Immunoglobulins, 978
Indirect dryer, 164
Infant nutrition, 969
Infrared spectroscopy, 141
Infusion, 452, 789
Ingredients, 262–265, 272, 274, 609, 611, 614
  fat, 263, 266–267
  flour, 262–265, 267
  gluten, 263–265, 267, 274
  salt, 266, 272
  sugar, 264, 266–267
  water, 262, 265–269, 271
Inhibitors, 587
Inorganic salts, 930
In-process sampling, 697
Instant noodles, 550, 552, 562
Institute of Medicine Food and Nutrition Board, 906
International Life Science Institute, 906
International Society of Beverage Technologists (ISBT), 433
  analytical procedure for bottler's carbon dioxide, 433
  quality guidelines for bottler's carbon dioxide, 433
Invertase, 188
Irish whiskey, 517
  distillation, 517
    new spirits, 517
    strong feints, 517
    strong low wine, 517
    weak feints, 517
    weak low wine, 517
Iron, 993, 998, 1007, 1009
Irradiation, 126, 857–858
Isoamyl acetate, 183, 185–186, 196, 475, 820
Isoamyl alcohol, 182, 196
Isoamyl alcohol acetyltransferase, 475
Isobutanol, 185–186, 472
Isobutyl acetate, 185–186, 856
Isobutyl alcohol, 182
Iso-butyric acids, 182
Isoflavones, 911, 915, 999, 1004, 1006
Isolated soy protein (ISP), 923
Isoleucine, 975
Isopenthyl acetate, 185
Isothermal heating system, 393
Iso-valeric acids, 182

Japanese whisky, 518
Juice concentration, 858
Juice extraction, 856

Juice post-extraction treatments, 856
Juice treatments
  thermal and non-thermal, 856

5-Ketododecanoic acid, 196
α-KA. *See* α-Keto acids
α-Keto acids, 472–473
α-Ketoglutarate, 476
α-Ketoglutarate dehydrogenase, 475
α-Ketoisocapric acid, 473
α-Ketoisovaleric acid, 473
α-Keto-β-methylvaleric acid, 473
Kava kava products, 926
Kefir manufacturing, 62
Ketones, 181, 209–210
Kettles, 26
Kettling, 297
Kimchi, 79
Kirsimen, 544
Kluyveromyces, 181
Kluyveromyces lactis, 187
Kluyveromyces marxianus, 185
Knife-cut noodles, 544
Konjac (Konjak) flour, 561
Konjac (Konjak) yam, 561
Krausen, 478
Krebs cycle, 476
Kuan mien, 544

α-Lactalbumin, 977
β-Lactoglobulin, 977
γ-Lactones, 720
δ-Lactones, 187
Lactase, 188, 284
Lactate, 715
Lactic acid, 181, 604, 636
Lactic acid bacteria (LAB), 362, 1023
  Lactobacillus sanfranciscensis, 363
  starters, 362
Lactic acid bacteria fermentation, 718
Lactobacilli, 157
Lactococcus casei, 478
Lactococcus lactis, 571
Lactoferrin, 959
Lactones, 187
Lactose (also lactate), 588, 597, 604, 612, 654, 714–715, 735, 982
Lactose intolerance, 1020
Lamiaceae, 208
Latin American white cheese, 648
  manufacture, 638

Laurel, 209
Lauric acid, 718
Lautering, 452
L-carvone, 210
L-cysteine, 861
LDL cholesterol, 922–923
LDPE. *See* Low-density polyethylene
Leaching, 150
Leavening agents, 289, 311
  air, 216
  baking powder, 289, 312
  baking soda, 289, 312
  sodium bicarbonate, 289, 312
  steam, 311–312
Lecithin, 313, 730
Legislative guidelines, 135
Lentinus edodes, 183
Leucine, 473, 477, 975
LEU4 gene, 475
Lg-ATF1 gene, 475
Leuconostoc, 181, 619
Limonene, 187, 210, 392, 394, 400–408, 411, 413, 852–853, 857–861
Limonin, 853
Linalool, 210, 852–853, 857–858
Linalool oxide, 857–858
Linalyl acetate, 210
Line extension, 133
Lipase (also lipolysis), 571, 573
Lipids, 445, 473–474, 476, 533–534, 549, 570, 726–727, 745, 752, 982–984, 1001–1002, 1004
  cholesterol, 990–993, 997, 999–1001, 1003, 1006
  fatty acids
    conjugated linoleic acid (CLA), 991, 993, 997
    monounsaturated (MUFA), 991
    polyunsaturated (PUFA), 991, 997, 1003
    saturated (SFA), 991
    trans, 991
  meat, 990–991
  oxidation, 997–999, 1005–1009
Lipid hydrolases, 854
Lipid oxidation, 858, 997–998, 1005–1009
Lipolysis, 726, 731, 732, 733
Lipoxygenase, 854, 856, 885
Liquid fermentation, 196, 197
Liquid(s), 290, 322, 406
  in cakes, 310–311

Live microbial food supplements, 920
Lobster, 284
Longxu (dragon whiskers), 543
Lot, 225
Low flavor impact, 121
Low-density polyethylene, 859
Lycopene, 916
Lyophilization, 167
Lysine, 353

1-Menthol, 187
2-Methyl butanol, 471
2-Methyl-1-propanol, 186
2-Methyl-3-furanthiol, 859, 861
2-Methylbutanoate, 856
2-Methylbutyl acetate, 781
2-Methyl-ethyl-butyrate, 185
3-Methylbutanal, 849
4-Mercapto-4-methylpentan-2-one, 853
5-Methylfurfural, 857
β-Myrcene, 852–853, 861
Macaroni, spaghetti, vermicelli, and noodles, 113
Mace oil, 211
Maceration, 211. *See also* Extraction with hot fat
Mad cow disease, 133
Magnesium, 921, 959
Maillard, 451–452, 457
Maillard browning, 301, 310, 318, 410
Maillard reaction, 9, 126, 287, 310, 352–353, 363, 366
  dried pasta, 329
  fresh pasta, 329
Major tropical fruits and products: banana, mango, and pineapple, 815–844
MAL gene, 469
Malate, 476
Malic acid, 854
Malt, 108–109, 133, 174, 295, 297, 301
Malt beverages, 108
Malt extract, 108, 295, 301
Maltase, 188, 449, 451, 469
Malting, 444, 450–451, 458
  germination, 508
  kilning, 458–459
  steeping, 449, 508
Maltol, 430
Maltose, 301, 356, 363, 373, 450
  metabolism, 451
  transporter, 469
Maltotriose, 450, 468, 470
  metabolism, 451
Mandarin juices, 857
Mango, 826–833
  antioxidant capacity, 821, 829
  classification, 826
    Indian, 826
    Indochinese, 826
  cultivation, 826
  flavor, 828
  harvest, 827
    climacteric, 827
    ethylene ripening, 827
  nutritional content, 828
  phenolics, 821, 836
  processed products and processing, 829–833
    aseptic-packed, 829–830
    chutneys, 829
    concentrate, 832, 851, 858, 859
    infused dried, 832
    juice, 829–832
    leather, 832
    other mango products, 833
    puree, 829–830, 832
  storage, 827
    chilling injury, 827
  supply, 827
Manufactured ice, 113
Manufacturing coding system, 254
Manufacturing of processed cheese
  blending, 600, 607
  cheese selection, 600
  cooking, 598, 607, 609
  cooling, 602, 609
  creaming, 607
  homogenization, 609
  packaging, 609–610
  pasteurization, 609
Margarine, 705, 707
  consumer margarine and spreads, 715, 721
    dairy spreads, 720
    low fat margarine, 720
    soft table margarine, 719–720
  crystallization, 710–711, 713, 715, 717, 719–720
    polymorphism, 709
  evaluation, 754, 768
  hippolyte mege mouries, 705
  industrial products, 715–716
    bakery margarine, 716

cake and cream margarine, 717–718
puff pastry margarine, 711, 717
shortenings, 716
ingredients, 707–709, 712–713, 720
antioxidants, 708–709
β-carotene, 708
citric acid, 709
color, 709
emulsifiers, 708–709, 711
fat phase and types, 707–709, 711, 713, 721–722
feef tallow, 717
hydrogenated, 708, 717–719, 721–722
interesterified fat, 721
lauric oils, 718
palm, 717–719
SFC profile, 707–708, 717–719, 721
flavor, 709, 718, 722
gelatine, 720
preservatives, 709
salt, 707, 709, 713
water phase, 707, 709, 711–713, 721
microbiological stability, 883
milk proteins, 709, 737, 744
pin rotor machine, 713, 715–717
processing and product trends, 722
processing methods, 721
drum chilling, 706
scraped surface heat exchanger, 706
processing values for residence time by margarine type, 714
resting tube, 713, 715
stabilizers, 720
trans fat, 719
Marjoram, 209
Market growth, 132, 950
Market maturity, 132
Marketer, 139–140, 145, 520
Mashing, 447–449, 453, 455, 464
Mass spectra, 141
Mass spectrometry, 141, 210, 217
Mass transfer limitation, 485
Maturation, 478–483, 485, 488–490
Maturation chambers, 870
MET gene, 480
Maturity, 872
Meat
composition, 994–997, 999, 1000, 1004, 1006–1007
processing, 994, 999, 1002, 1007, 1009
Meat packaging plants, 89
Meat products, 63, 89–91, 990–1010
composition, 994–997, 999
formulation, 1000, 1003–1007
reformulation, 994, 999–1000
types
cooked ham, 1003, 1005
fermented sausages, 1003
Frankfurter, 1003, 1005
ground beef, 1001, 1003
pork liver pâté, 1003
restructured beef, 1005–1006
Mechanical operations, 835, 882–883
cutting and/or slicing, 882–884
peeling, 882–883
Medium flavor impact, 121, 128
Mee, 540
Mental association, 144
Menthol, 187, 210
Meringue method, 313, 315
Methional, 861, 936
Methyl anthranilate, 855
Methyl octanoate, 857
Methyl-3-buthanol, 186
Methylfuraneol, 855
Mi fen, 540
Microbial fermentation, 193–194
Microbial inspection, 696
Microfiltration, 200, 856
Microorganisms, 170–174, 179–189, 225, 233, 235
Microwave and radio frequency processing, 38–39
Microwave foods
lack of crispness and browning, 409–410
Microwave heating, 393, 409–411
Microwave heating of flavor-formulated dough, 409–413
Mie, 540
Mien (Mian, mein), 540
Mikania glomerata, 166
Milk, 618, 653, 713–738
different breeds, 727
flavor, 713–738
flavor characterization, 724–737
flavor production reactions, 714–721
milk composition, 570, 574, 576, 583, 587, 588
raw, 570–571, 575–576, 584
specific flavors, 731–734
specific off-flavors, 731–734
taste compounds, 734–737
Milk, chemical composition of, 972

Milk, functional properties of, 973
Milk, mineral composition, 982
Milk, nutritional characteristics, 972
Milk constituents, functional properties of, 973–986
Milk fat, 979–981
  conjugated linolenic acids, 979
  constituents, 978
  crystals, 734–735, 738, 739, 741, 744
  fatty acids, 979, 980
  fatty acid profile, 979–980
  phospholipids, 981
  physiological effects, 973, 976, 979
Milk fat globule membrane (MFGM), 726–728, 730, 733–735, 738
Milk lipids, 726–727
Milk physical structure, 973
Milk products, 713–738
  flavor, 713–738
  flavor characterization, 724–737
  flavor production reactions, 714–721
  specific flavors, 731–734
  specific off-flavors, 731–734
  taste compounds, 734–737
  thermally-induced changes, 715
Milk proteins, 971
  caseins, 974
    peptides, 974
  whey/serum proteins, 977
    peptides, 979
Milling, 448, 449
Minerals, 921, 942, 998
Misbranding, 137
Miscellaneous food preparations and kindred, 111–115
Mislabeled products, 699
Miso production, 75
Mixers, 319
  continuous mixers, 320
    AMF mixer, 321
    baker Perkins homogenizer, 321
    Oakes continuous automatic, 320
    Oakes marshmallow, 320
  vertical dough mixers, 319
    planetary, 319
    pressure bowl, 319
Mixing methods–shortened cakes, 315, 318
  conventional meringue, 315
  conventional sponge, 315
    pastry blend, 315
    single stage, 315
  creaming, 314–315
Mixing, 24, 236, 291, 296, 299, 313–316, 319–320
MMS, 857. *See also* S-Methylmethionine sulfonium
Model flavor-dough system, 392
Modeling, 272–274
  artificial neural network, 272
  CFD, 272
  empirical model, 272–273
  kinetic model, 272
  mapping technique, 273
  mathematical model, 272, 274
Modified atmospheric packaging (MAP), 28
Modified atmospheric storage (MAS), 28
Modified gas atmosphere, 28
Moisture, 595, 597, 604, 616
Moisture content, 216
Moisture migration, 390, 409
Molding starch and trays, 698
Monoglycerides, 287, 657, 708
Monosodium glutamate, 123
Monoterpene alcohols, 852
Monounsaturated fatty acids, 720
Morchella, 183
Morels, 183
Mouthfeel, 287, 301
Mozzarella, 607–608, 734
Muffins, 280, 284–287
  bread-type, 286
  cake-type, 285–286, 292
  English, 280
  evaluation, 292, 299
  quick-bread, 280
Mung bean threads, 559, 561
Mushroom flavors, 183
Musts, 855
Mycotoxins, 217
Myrcene, 210, 852, 859

Naringin, 853, 856
National Soft Drink Association, 421
Natural, processed, and imitation cheese, 92
Neotame, 310
Neral, 852
Neryl acetate, 853
Neurospora, 186
NFDM, 310, 619, 697
Nisin, 604
Nitrite, 1002, 1005
Nonanal, 857

Nonenzymatic browning, 613, 861
Non-fat dry milk (NFDM), 310, 605, 697
Nonstarter lactic acid bacteria (NSLAB), 572, 589
Nootkatone, 853, 858
NSLAB, 572
Nuclear magnetic resonance, 141–142
Nucleotides, 129
Nutmeg oil, 211
Nutraceutical, 123, 905
  biological effects, 914–921
  definition, 906
  dietary guidance, 929
  effects on major chronic diseases, 913
  examples, 906, 911
  public education, 929, 931
  regulation of, 927–928
  safety issues, 905, 924
  sources in nature, 914–922
Nutrition, 610
Nutrition Labeling Education Act (NLEA), 283, 940, 949
Nutritional characteristics, milk constituents, 972
Nutritional labeling, 255
Nylon, 857–858

1,5-Octadien-3-one, 939
1-Octen-3-ol, 729, 939
1-Octen-3-one, 730, 854, 939
β-Ocimene, 856
Oakes continuous automatic mixer, 320
Oakes marshmallow mixer, 320
O-aminoacetophenone, 855
Oats, 922, 1021, 1024, 1026
Obesity, 280, 913–914, 927
Ocean perch, 930
Octanal, 853, 857, 861
Octanol-3, 186
O-ethers, 210
Off-flavor, 745
Ohmic and inductive heating, 39
Oil recovery under pressure, 154
Oily-noodles, 552, 555
Oleo-resins, 212
Omega-3 fatty acids, 907–908, 924
Onion, 208, 218
On-line flavor concentration measurement methodology, 393–400
OR. *See* Oleo-resins
Organic acids
  biosynthesis, 476
Orange juice, 847–861
Orangeade, 135
Ordinary (itohiki) natto, 73–74
Oregano, 208–209, 212
Organic acids, 852, 874
Organic Foods Production Act of 1990, 284
Oscillating magnetic fields, 41
Osmotic dehydration, 871
Osteoporosis, 920
Ovens, 322–324
  band, 323
  reel, 322
  rotary rack, 323
  travelling, 323
  tunnel, 324
Overmixing batter, 315
Oxygen, 448–449, 454, 473–474, 481

2-Phenylethanol, 471–472, 849, 852
2-Phenylethyl acetate, 474–475
3-Phenylpyruvate, 473
2,3-Pentanedione, 477–478, 488
6-Pentyl-2-pyrone, 187
6-Pentyl-α-pyrone, 187, 195–196
6-PP. *See* 6-Pentyl-α-pyrone
α-Pinene, 174, 848, 852
β-Pinene, 849
n-Propanol, 182, 471–472
p-1-menthene-8-thiol, 851, 853, 855
Package (also packaging), 583, 585, 590, 609, 627
Packaging, 42–43, 254, 270, 274, 292, 298, 888
  anaerobic conditions, 572
    anaerobic pathogenic microorganisms, 888
    fermentative processes, 888
  carbon dioxide, 268, 271, 274
  edible film, 271
  hurdle technology, 271
  MAP, 271
  moisture, 272
  oxygen, 888–889
  preservatives, 271–272
  retrogradation, 271
  shelf-life, 270–271, 273–274
  staling, 271
  water activity, 267, 271–272
Packaging materials, 53, 252
  glass, 228
  laminates (paper-based), 43
  metal can, 43

Packed-bed, 199
Packed columns, 158
Packing, 437
Paneer, 645–648
  defects, 647
  manufacture, 647
  microstructure, 648
  packaging, 646
  yield, 647
Pans, cake, 316
Parsley, 208–209
Partial coalescence, 660
Particle size, 216
Pasta, dried, 330
  cooking quality, 333
  drying, 338
  extrusion, 335
  heat-damage, 352. *See also* Maillard reaction
  process innovations, 335
  quality characteristics before cooking, 331, 335
  raw materials, 343–344
Pasteurization, 570, 574–576, 585, 588, 622–623, 728, 857, 858
Pastry blend method, 315
Pathogenic bacteria, 8, 962, 964
  Campylobacter jejuni, 8–9, 884
  Cyclospora cayetanensis, 884
  Escherichia coli, 8, 880
  Listeria monocytogenes, 8, 880
  Salmonella enteritidis, 961
  Salmonella spp., 880, 886
  Shigella spp., 884
Pathogens, 217, 570, 575, 586
Peak area, 395
Pears, 853–854
Pectinase, 188, 856
Pecylomyces, sp., 186
Penicillium, 186, 188
Penicillium decumbens, 183
Penicillium roquefortii, 181, 183
Pentane-2,3-dione, 182
Pentanol, 186
Pentonases, 188
Pentosans, 445, 451
Pepper, 213–215, 218
Peptidases, 188
Peptides, 953, 958, 959
Perfumery industries, 148
Pervaporation, 202
Pest, 225
  control, 229
Pesticide residues, 217
PET. *See* Polyethylene terephtalate
pH, 232–233, 235, 309, 576, 578, 581–582, 590, 600, 608, 619, 621, 623–625
Phenyl ethanol, 186
Phenyl ethyl alcohol, 186
Phenylacetaldehyde, 855
Pho, 540
Phosphate, 729
Phospholipids, 654, 726–728, 730
Photoionization detection, 393–396
Phytate, 1006, 1009
Phytochemicals, 905
  areas of future research, 905, 913
Phytoestrogens (isoflavones), 915, 923
Phytosterols, 923
Pickled fruits and vegetables, vegetable sauces and seasonings, and salad dressing, 96–97
Pickles, 16, 78–79
PID. *See* Photoionization detection
Pineapple, 833–842
  antioxidant capacity, 821
  aroma profile, 838
  classification, 833
    commercial varieties, 834
    Del Monte gold extra sweet, 833
    Smooth cayenne, 833
  consumption, 836
  cultivation, 833
  flavor components, 836
  Ginaca machine, 837, 839
    chunks, 837, 839
    juice, 837–839
    other pineapple products, 838–842
    pineapple Slices, 837–839
    tidbits, 839
  harvest, 833
    brix, 833
    de-crowning, 835
    non-climacteric, 833
    plantation, 833, 835
  nutritional composition, 836
  phenolic content, 821, 836
  processed products, 837–839
  storage, 833
  supply, 834–835
Piperine, 212, 217
Plant sterols, 1006
Plant, 225
  construction and design, 227

Plasmin, 727
Plate freezers, 30
Pleurotus, 183
Plumbing, 230
Polyamines, 994
Polycyclic aromatic hydrocarbons, 1007
Polydextrose, 310
Polyelectrolyte polymer brush, 653
Polyethylene terephtalate, 859–860
Polymorphism, 709
Polyphenolic compounds, 918
Polyphenols, 743
Polypropylene, 859–860
Polyunsaturated fatty acids, 924, 945
Populus nigra, 167
Postmenopausal incidences, 923
Potassium, 800, 804
Potassium sorbate, 857–858
Potato chips, corn chips and similar snacks, 112–113
Potentiator, 128–129
Poultry slaughtering and processing, 90–91
PP. *See* Polypropylene
Prebiotics, 920, 966
Prepared feed and feed ingredients for animals and fowls except dogs and cats, 101
Prepared flour mixes and doughs, 98–99
Prepared fresh or frozen fish and seafoods, 111–112
Pressing, 160, 561
Probiotics, 920, 958, 960, 963, 1004, 1005
  health benefits, 961, 966
    anticarcinogenesis, 965
    anti-inflammatotory, 964
    lactose digestion, 963
    prevention of diarrhea, 963
    prevention of infectious diseases, 964
    reduction in serum cholesterol, 965
    stimulation of immune system, 964
    urinary tract infections, 965
  requirements, 958
Processing and preservation techniques, 24–41
Product decline, 132
Product development, 132–145
Product flowcharts, 11–12
Product formulations, 11
Product introduction, 132
Product life cycle, 135
Proline, 975
Proofing, 297
Propanoic acid, 849
Propanol, 186
Propionibacteria, 181
Propionic acid, 271
Propyl acetate, 186
Propyl butanoate, 856–857
Proteases, 188, 600, 854
Protein, 930, 991, 996, 1001, 1004
  amino acids, 991
  plant-based, 1004
Proteolysis, 572–573, 576, 584, 605, 976
Proving, 265–268, 274
  fermentation, 266–268
  humidity, 268
  proving temperature, 268
  volume expansion, 268
  yeast, 262–263, 266–268, 271, 273
Provolone, 188
Pseudocereals, 1022–1023, 1025
  amaranth, 1023–1024
  buckwheat, 1023
  quinoa, 1023
Psychrotrophic bacteria, 570
Psyllium, 922
Pulsed electric field processing, 125
Pulsed electric fields (PEF), 40
Pulsed light technology, 41
Pulsed X-ray, 42
Pumping, 23
Pyrazine, 187, 400–401, 406
Pyroglutamate, 476
Pyruvate, 471, 476

Quality control operation(s), 225, 232
Quality, 421, 425
Quantitative descriptive analysis, 143
Quark, 636
Queso blanco, 648
Quick mix methods, 315
Quinoa, 1023

Radiation heat, 322
Raisins, 12
Rajbhog, 643
Ramen, 550
Rasmalai, 643
Rasogolla, 639–640
  cooking medium, 641
  packaging, 646
  sugar syrup, 641
  yield, 642

Raw materials handling, 20
Rectification, 159
Reduced-fat, 311, 573, 575
Reel oven, 322
Refrigerators, 27, 79
Rehydration, 868
Retarded dough, 69
Retorts (pressure cookers), 26
Reverse R & D, 133
Rework, 226
Rhizopus, 76
Rhizopus oryzae, 195
Rice milling, 528
Rice noodle (fen), 540, 556
Rice sticks, 557
Rice, 523
  bran, 533
  brown, 526
    minerals, 526
  flour, 531
    breads, 532
      enzymes, 532
      hydrocolloids, 532
  hulls, 533
  parboiled, 526
  processing, 525
  vitamins, 526–527, 529
    enrichment, 529
Rice-based Asian noodles, 556–558
Ricinoleic acid, 172
Ricinus communis, 197
Ricotta cheese, 638
  manufacture, 638
Ripening, 569, 572, 576, 578, 580, 584, 588, 880–881
  climacteric fruits, 881
  ethylene, 881–884, 889
Roasted coffee, 112
Roasters, 26
Rocking-drum bioreactor, 199
Romano, 188
Rosemary, 209, 212
Rotary rack oven, 323
Rotating drum, 199
Rue oil, 209

α-Sinenasal, 852
β-Sinenasal, 852
Saccharin, 310
Saccharomyces carlsbergensis, 183
Saccharomyces cerevisiae, 175, 183
Safe moisture level, 226
Safety, 575, 583–584, 586
Safrole, 211
Sage, 212
Sai fen, 559
Saimen, 545
Sales, 618, 633
Salmonella, 8, 217
Salt, 290, 296, 569, 572, 583, 585, 618–620. *See also* Sodium, Salting
  Salt in moisture ratio, 583
Salted and roasted nuts and seeds, 106
Sampling schedule for color containing products, 253
Sandesh, 639–640
  physico-chemical attributes, 644
Sanitation, 229, 240
Sanitizing, 226, 229
  agents, 228
  food-contact surfaces, 229
  non-food contact surfaces, 229
Sanitizing treatments, 881
  chlorine, 881
  chlorine dioxide, 881
  hydrogen peroxide, 798, 885
  organic acids, 880
  ozone, 885
  ultraviolet light, 885
Sapid compounds, 209–212
Sassafras root oil, 211
Sauerkraut, 78
Sausages
  basic steps in dry sausage processing, 65
Sausages and other prepared meat products, 89–90
Savory noodles, 552
Scaling, 285, 295, 297
Scottish blended whisky, 516
  patent still (Coffey still), 515
    analyzer, 515
    rectifier, 515
  Scottish grain whisky, 515
Scottish malt whisky, 508
  angel's share, 513
  Bourbon, 513
  Campbeltown, 515
  cask, 513
    charring, 513
  distillation, 510
    congeneric, 512

demisting test, 512
feints (tail), 513
feints receiver, 513
foreshot (head), 512
hearts (middle cut), 513
low wine, 512
low wine charger, 512
low wine still, 512
new pot (new spirit), 513
spent lees, 513
spirit, 513
spirit receiver, 513
wash still, 512
draff, 510
dunnage warehouse, 513
highland malts, 515
Islays, 515
kiln, 508
lowland malts, 515
malting, 508
germination, 508
steeping, 508
maltster, 509
mash tun, 509–510
peat, 509
pot still, 510
condenser, 512
lyne arm, 510
worm, 512
sherry, 513
wash, 515
washback, 510
wort, 510
Scrap candy, 699
Scraped-surface freezers, 31
Seasoner, 128
Seasoning blends, 219
Secondary metabolism, 180
Selenium, 924, 993
Semolina, 329–330, 333
rheological characteristics, 332
Seniors nutrition products, 969
Sensorial simulation, 145
Sensory analysis, 143
Separating, 22
Separation, 622, 664–665
Separation technique, 157
Septoria nodorum, 187
Sesquiterpene, 210, 852
S-ethers, 210
Sewage disposal, 230
Sex odor, 123
SFA1 gene, 473
Shallot, 208
Shelf-life, 287, 298, 584, 600, 611
Shellfish, 21, 29
Shiitake, 183
Shirataki, 561
Shortened style cakes, 314
Shortening, 311
Shortening, table oils margarine and other edible fats and oils, not elsewhere classified, 108
Shrimp, 30–31
Silicon carbide, 489
Single stage (dump method), 315
Single-service articles, 229
Small entity compliance guide (SECG), 929
Smell, 207
S-methylmethionine sulfonium, 855
SmF. *See* Submerged fermentation
SNIF/NMR, 215
Soba, 377
Soda pops, 420. *See also* Carbonated soft drinks
Sodium, 729, 737, 895
Sodium aluminum sulfate, 312
Sodium chloride, 290
Soft drinks, 420. *See also* Carbonated soft drinks
Softening, 777
calcium chloride, 786
calcium lactate, 888
polygalacturonase, 784
proteolytic enzymes, 883
Solid fermentation, 197–198
Solid-liquid extraction, 150
Solid-phase micro extraction, 152
Solid-state fermentation, 197–198
Solvent extraction/AEDA, 155, 212
Somatic cells, 571
Somen, 541–542, 544
Sorbic acid, 604, 614
Sorghum, 1025–1026
Sour milk manufacturing, 60–62
Sourdough, 361, 367, 1023–1024
fermentation, 1023
starter cultures, 572
types, 361
Soxhlet extractor, 153
Soy sauce, 73
Soybean oil mills, 106–107

Soybeans, 922–923
Spearmint oil, 210
Spent grains, 487
Spice blends, 219
Spice trade, 213–215
Spices, 205–220
  in the food industry, 217–219
Sponge cake, yellow, 318
Sporidioolus salmonicolor, 201
Sporobolomyces odorus, 187
Sports nutrition products, 969
Spray-drying, 201–202
Spreading, 719
SSF. *See* Solid-state fermentation
Sniff testing, 143
St. John's wort, 926–927
Stabilizer, 620
Starch, 449–450
Starch noodles, 559
Starch-based noodles, 559–561
Starter culture. *See* Culture
Steam, 312
Steam distillation, 160–162, 211, 410–411
Steamed bread (Mantou), 70–71
Stinky tofu, 75–76
Stirred aerated bed, 199
Sterols, 333
Storage, 667, 880, 887, 889, 891
Storage condition, 574, 667, 686, 691
Strawberries, 793–804
  average production, 795
  demand, 795
  physicochemical, phytonutrients, and nutritional qualities, 808–811
    anthocyanins, 798
    antioxidant capacity, 799, 808–809
    antiproliferative properties, 799
    nutritional quality, 800
    pectins and cell wall enzymes, 797
    phenolic compounds, 798
    strawberry flavor, 797
    sugars and organic acids, 796
  strawberry products, 800–804
    freeze dried, 804
    frozen, 800
    infused and stabilized, 803
    infused dried, 804
    strawberry juice, 801
    strawberry puree, 800
  strawberry varieties and postharvest handling, 795–796
Strawberries and blueberries: phytonutrients and products, 793–812
Strecker aldehydes, 464
Strecker degradation, 185, 861
Streptococci, 181
Streptococcus thermophilus, 961
Streptococus lactis, 184
Stripping, 149, 525
Stripping using water vapor, 149
Submerged fermentation, 196
Succinic acid, 885
Succinate dehydrogenase, 476
Sucrose, 286, 468, 852–853
Sufu (fermented soy cheese), 75–76
Sugar, 286, 431, 432, 926
  beet sugar, 104, 431
  brown, 310
  cane sugar, 103–104, 252, 308
  granulated, 314
  handling and treatment at beverage plant, 432
  liquid sucrose, 431, 435
  medium Invert sugar, 431–432, 435
Sugar and confectionery products, 103–106
Sugar replacers, 266, 281, 287, 302
Sugar-free coating, 689–690
Sulphite, 480–481
Sulfite reductase, 481
Supercritical extraction, 167
Supercritical fluid extraction, 152–154, 162
Supervision, 227
Supplies, 425–426
Sweet fennel, 210
Sweeteners, 286, 295, 429–432
  corn syrup, 286, 295
  dextrose, 295
  fruit juice, 286
  honey, 286, 295
  maple sugar, 286
Swiss cheese manufacturing, 55, 57
Symbiotics, 920
Synbiotics, 966
Syneresis, 666–667

2-*Trans*-hexenal, 858, 860
α-Terpineol, 173, 857, 861
γ-Terpinene, 853
TAG. *See* Triacylglycerol
Tangerine flavor, 853

Tanks, 26
Tarragon, 210, 218
Taste, 207
Tea, 918
Tempe (tempeh), 76–77
Temperature, 880–881, 891–892
Tempering of chocolate, 684–686
Terpenes, 187, 210, 852
Terpenyl acetate, 210
Terpin-4-ol, 857
Terpineols, 210
Tert-butylbenzene, 392–408
Texture, 293
T_g. *See* Glass transition temperature
Thermal degradation, 857
Thiamin, 861
Threonine, 478, 484
Thyme, 167, 209
Thymus vulgaris, 167
Tiquira, 186
Titratable acidity, 619, 624
Tomatoes, 916
Trace elements, 922
Trametes odorata, 187
Trans-2-nonenal, 481, 855
Trans-2-hexenal, 856, 860
Transamination, 472
Transfer of heat, 322
Transglutaminase, 1027
Transport, 667
Trans-resveratrol, 919
Travelling ovens, 323
Tray, 199
Tray columns, 158
Trends, 273
  automation, 274
  frozen dough, 273–274
  frozen par-baked products, 273
  functional food, 273
  health, 262, 273
  optimal control, 274
Triacylglycerol, 653
Trichoderma, 188
Trichoderma harzianum, 187, 195
Trichoderma viridae, 183, 185, 187, 196
Trichosporon, 181
Triglyceride. *See* Triacylglycerol
Triglycerides, 209, 718
Trimethylamine, 940
Triticum, 1021
Trub, 447, 466–467
Truffles, 183
Tuber sp., 183
Tunnel ovens, 26, 324
Turmeric, 217
Tryptophol, 471–472
Tyrosol, 471–472

Udon, 543, 545
UHT treated milk, 743
UHT-treatment, 735
Ultrafiltration, 200, 575
Ultrasound, 42
Ultrastructure
  buckwheat, 376–378
  dried pasta, 333
  fresh pasta, 339
Ultraviolet light, 41
Umbelliferae, 208
Umbelliferous fruits, 122
Unit operations, 13, 20–24
U.S. Code of Federal Regulations (CFR), 224
U.S. Compressed Gas Association (CGA), 433
  quality verification levels, 433
U.S. FDA Food Additives Status List (FASL), 252
U.S. FDA United Registartion and Listing System ((FURLS), 246
U.S. Food and Drug Administration (FDA), 224
U.S. Occupational Safety and Health Administration (OSHA), 434
U.S. Standard Industrial Classification (SIC), 87
  SIC Major Industrial Group 20 (foods and kindred products), 88
  SIC Manual, 87

4-Vinylguaiacol, 857
p-Vinylguaiacol, 861
Vacuum steam-stripping, 154
Valencene, 852–854
Valencia orange juice, 861
Valencia oranges, 853
Valeric acid, 182
Valine, 174
Vanillin, 197, 513
Vaporization, 162
Vapor-liquid equilibrium, 160
Vegetable oils, 997, 1001, 1003

Vegetable oil mills except corn, cottonseed, and soybean, 107
Vicinal diketones, 182, 477–479
  biosynthesis, 471
Violations in confectionery products, 700
  choking hazards, 700
  filth contaminations, 700
  honey with chloramphenicol, 701
  undeclared ingredients, 700
Virgin olive oil, 753, 758
  carotenoid, 761–762
  chlorophyll, 765–766
  extraction process, 753
  phenols, 765–766
  quality, 763
  tocopherol, 761
  volatiles, 765
Viscosity of chocolate, 683–684
Vital wheat gluten, 295
Vitamins, 921, 984, 993, 997–998, 1009
  fat-soluble vitamins, 921
  water-soluble vitamins, 921
VLE. *See* Vapor-liquid equilibrium
VO. *See* Volatile oil
Volatile oil, 148–149, 212
Volatiles, 828
Volume, 289, 317, 318
Volvariella volvacea, 183

Washing, 582, 625–626
Water, 425–428, 621, 625
  purification treatments, 431
    conventional lime treatment systems (CLTS), 427
    ion exchange, 428
    membrane technology, 427–428
  quality, 427, 611
  supplies, 425–426
Water activity, 226, 232, 287, 291, 302
Water radiolysis, 126
Water supply, 230
Weed off-flavor, 745
Weight reduction products, 968
Wet corn milling, 100–101
Wheat flour, 66
Wheat-based Asian noodle, 542–554
  chemical properties comparison, 542
Wheat-salted Asian noodles, 543–545
  flat noodles, 544
  general manufacturing scheme, 545
  thick noodles, 543
  very thin noodles, 543
  wide flat noodles, 544
Whey, 570–572, 574, 576, 578–584, 590, 596, 602, 608, 611, 622, 624–626, 630–631
Whey protein, 653–654
Whisky (whiskey), 507
  alchemist, 507
  aqua vitae, 507
  Scotch whisky, 513
  uisge beatha, 507
  usquebaugh, 508
  water of life, 507
White, unripened cheeses, 637
WHO/FAO, 1021
Whole spices, 218
Wine, 182, 206, 918
Wines, brandy and brandy spirits, 109
Woodchips, 490
Work-in-process, 234
World Health Organization (WHO), 11, 302, 1021
Wort, 443
  acidification, 458
  aeration, 447
  aldehydes, 458
  boiling, 447, 452, 455–456
  carbohydrates, 467
  clarification, 447, 457, 464
  cooling, 447, 456, 459, 466
  high-gravity, 468, 474
  separation, 448–449, 452, 454

Xi mien, 543
X-ray detector, 216

Yam noodles, 561
Yang-chun mien, 544
Yarrowia lipolytica, 197
Yeast, 295–296, 356
  alloploid, 470
  aneuploid, 470
  cell membrane, 468
  compressed, *Saccharomyces cerevisiae*, 361
  growth kinetics, 468
  immobilized, 186, 190
  lipid vesicles, 476
  plasma membrane, 470, 474, 484
  polyploid, 469–470

species in sourdough, 361, 363
Yellow-alkaline wheat-based Asian noodles, 545–546
Yield, 570–571, 574–576, 579–580, 584, 619–621, 624, 628
Yi-mien, 545
Yinsi (silver threads) noodle, 543
Yogurt, 12, 57, 59
common gums used, 60
flavors used, 60
permitted colorings, 61
standards, 61

Zinc, 473, 993, 1009

Contents

Health, Meat, Milk, Poultry, Seafood, and Vegetables

PREFACE ix

CONTRIBUTORS xiii

PART B: FOOD PRODUCTS MANUFACTURING (CONTINUED) 1

Section XII: Health Ingredients and Health Products Development for Preventing or Treating Human Diseases 3

46. Bioactive Peptides from Food Proteins 5
Hannu Korhonen and Anne Pihlanto

47. Lipid-Soluble Vitamins: Nutritional and Functional Aspects 39
Antonio Pérez-Gálvez and María Isabel Mínguez-Mosquera

48. Phytochemicals in Mediterranean Diet: The Interaction Between Tomato and Olive Oil Bioactive Compounds 55
Rita Pernice, Paola Vitaglione, Raffaele Sacchi, and Vincenzo Fogliano

49. Functional Microbes: Technology for Health Foods 67
Maria Saarela and Tiina Mattila-Sandholm

50. Enterococci and Dairy Products 85
Giorgio Giraffa

51. Fermented Milk: Health Benefits Beyond Probiotic Effect 99
Todor Vasiljevic and Nagendra P. Shah

52. Application of Functional Foods and Nutraceuticals in Allergic Disorders: Principles and Potential **117**
Venu Gangur

53. Functional Foods, Herbs, and Aging **135**
Carlos Kusano Bucalen Ferrari

54. Functional Foods and Gastrointestinal Disorders **153**
Jude Ryan, Shomik Sibartie, Liam O'Mahony, and Fergus Shanahan

55. Herbs in the Management of Diabetes Mellitus with an Emphasis on Ginseng **175**
Vladimir Vuksan and John L. Sievenpiper

56. Functional Foods and Minerals: Calcium **201**
John J.B. Anderson

Section XIII: Meat and Meat Products **215**

57. Thermal Processing **217**
Isabel Guerrero Legarreta

58. Ham **233**
Fidel Toldrá

59. Sausages **251**
Fidel Toldrá and Milagro Reig

60. Fermented Meat Production **265**
Fidel Toldrá

61. Processed Pork Meat Flavors **281**
Fidel Toldrá and Mónica Flores

62. Sensory Quality of Meat Products **303**
Fidel Toldrá and Mónica Flores

Section XIV: Milk and Milk Products **329**

63. Processing Quality Fluid Milk Products **331**
James V. Chambers and Somjit Surapat

64. Milk Composition, Physical and Processing Characteristics **347**
Ramesh C. Chandan

65. Genetics and Milk Production **379**
Stephanie Clark and Michael J. Costello

66. Flavored Milks **403**
Kayanush J. Aryana

67. Fermented Milks Popular in Europe and North America **411**
A.Y. Tamime and R.K. Robinson

68. Fermented Milk in Asia 431
Sardar Iftekharudin Ahmed and Jutatip Wangsai

69. Goat Milk, Its Products and Nutrition 449
Young W. Park and George F.W. Haenlein

70. Bioactive Peptides in Dairy Products 489
Marco Gobbetti, Fabio Minervini, and Carlo Giuseppe Rizzello

71. Science and Technology of Sour Cream 519
Wilfried Rieder

72. Dairy Protein Hydrolysates 537
Ifendu A. Nnanna and Chao Wu

73. The Manufacture and Applications of Casein-Derived Ingredients 557
John E. O'Connell and Cal Flynn

74. Ice Cream and Frozen Desserts 593
Arun Kilara and Ramesh C. Chandan

75. Frozen Novelties 635
Jonathan Hopkinson

76. Yogurt 647
Lisa Pannell and Tonya C. Schoenfuss

77. Infant Formulas 677
G. Boehm, C. Beermann, G. Georgi, and B. Stahl

78. Biochemical Processes in the Production of Flavor in Milk and Milk Products 715
Tanoj K. Singh, Keith R. Cadwallader, and MaryAnne Drake

Section XV: Poultry Products 749

79. Poultry Marination 751
Edith Ponce-Alquicira

80. Poultry Sausages 773
Alfonso Totosaus and M. Lourdes Pérez-Chabela

81. Flavor of Fresh and Frozen Poultry 781
Edith Ponce-Alquicira

82. Edible Packaging for Poultry and Poultry Products 795
Baciliza Quintero-Salazar and Edith Ponce-Alquicira

Section XVI: Seafood 815

83. Frozen Seafood Products Description 817
Nanna Cross

84. Processing Frozen Seafoods **855**
Shann-Tzong Jiang and Tung-Ching Lee

85. Shellfish Freezing **893**
Athapol Noomhorm, Mukku Shrinivas Rao, and Punchira Vongsawasdi

86. Processing Formulated Fish and Fish Products **915**
Jamilah Bakar

87. Flavorants from Seafood Byproducts **931**
Rosa Jonsdottir, Gudrun Olafsdottir, Sigurður Hauksson, and Jon Magnus Einarsson

88. Seafood Processing: Basic Sanitation Practices **947**
Nanna Cross

89. Sustainable Intensive Aquaculture **969**
C.G. Carter

Section XVII: Vegetable Products **983**

90. Frozen Vegetables and Product Descriptions **985**
Nanna Cross

91. Frozen Avocados **997**
Rosa María Galicia-Cabrera

92. Frozen French Fried Potatoes and Quality Assurance **1013**
Nanna Cross

93. Paprika Production: Current Processing Techniques and Emerging Technologies **1031**
María Isabel Mínguez-Mosquera, Manuel Jarén-Galán, Dámaso Hornero-Méndez, and Antonio Pérez-Gálvez

94. Pickles Manufacturing in the United States: Quality Assurance and Establishment Inspection **1045**
Nanna Cross

95. Soymilk and Tofu Manufacturing **1063**
Sam K.C. Chang

96. Tomato Processing **1091**
Rosa María Galicia-Cabrera

97. Dried Tomato **1109**
Raquel Pinho Ferreira Guiné and Maria João Barroca Dias

VOLUME 2 INDEX **1123**

VOLUME 1 CONTENTS **1155**

VOLUME 2 CONTENTS **1159**

CUMULATIVE INDEX **1163**

Part B

Food Products Manufacturing

Continued from Volume 1.

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

Section XII

Health Ingredients and Health Products Development for Preventing or Treating Human Diseases

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

46

Bioactive Peptides from Food Proteins

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46.1 Introduction 5
46.2 Functionality of Bioactive Peptides 6
46.3 Production of Bioactive Peptides 9
46.3.1 Enzymatic Hydrolysis 9
46.3.2 Microbial Fermentation 12
46.3.3 Enrichment of Bioactive Peptides 17
46.4 Occurrence of Bioactive Peptides in Foods 18
46.4.1 Milk and Dairy Products 18
46.4.2 Eggs 21
46.4.3 Meat 22
46.4.4 Fish 22
46.4.5 Plants 24
46.5 Biological Effects of Bioactive Peptides 25
46.6 Future Applications of Bioactive Peptides 28
References 29

46.1 INTRODUCTION

The role of proteins as physiologically active components in the diet is being increasingly acknowledged. Many of the proteins which occur naturally in raw food materials exert their physiological action either directly or upon enzymatic hydrolysis *in vitro* or *in vivo*. In recent years it has been recognized that dietary proteins provide a rich source

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of biologically active peptides. Such peptides are inactive within the sequence of the parent protein and can be released in three ways: (1) through hydrolysis by digestive enzymes, (2) through hydrolysis of proteins by proteolytic microorganisms, and (3) through the action of proteolytic enzymes derived from microorganisms or plants. It is now well established that physiologically active peptides are produced from several food proteins during gastrointestinal digestion and fermentation of food materials with lactic acid bacteria. The state-of-the-art of bioactive peptides derived from various dietary proteins has been reviewed in many recent articles (Clare and others 2003; FitzGerald and Meisel 2003; Korhonen and Pihlanto 2003a; Pellegrini 2003; Pihlanto and Korhonen 2003; Li and others 2004). Once bioactive peptides are liberated, they may impart in the body different physiological effects on, for example, the gastrointestinal, cardiovascular, endocrine, immune, and nervous systems. For this reason, the potential of distinct dietary peptide sequences to promote human health by reducing the risk of certain chronic diseases and boosting natural immune protection has aroused a lot of scientific interest over the past few years. These functions may be attributed to numerous known peptide sequences exhibiting, for example, antimicrobial, antioxidative, antithrombotic, antihypertensive, and immunomodulatory activities (FitzGerald and Meisel 2003; Korhonen and Pihlanto 2003a). The activity is based on their inherent amino acid composition and sequence. The size of active sequences may vary from two to 20 amino acid residues, and many peptides are known to reveal multifunctional properties (Meisel and FitzGerald 2003). At present, milk proteins are considered the most important source of bioactive peptides, although corresponding peptide sequences have been found in other animal and plant proteins as well (Schanbacher and others 1998; Meisel 1998, 2001; Clare and Swaisgood 2000; Korhonen and Pihlanto-Leppälä 2001, 2004; Darragh 2002; Matar and others 2003; Korhonen and Pihlanto 2003b; FitzGerald and others 2004; Silva and Malcata 2005).

This chapter reviews the current knowledge about bioactive peptides derived from various food proteins, with emphasis on their production, natural occurrence in food products and potential applications in functional foods.

46.2 FUNCTIONALITY OF BIOACTIVE PEPTIDES

Dietary proteins are traditionally known to provide a source of energy and amino acids essential for growth and maintenance of various body functions. In addition, they contribute to the physiochemical and sensory properties of protein-rich foods. Over the last two decades, food proteins have gained increasing value due to the rapidly expanding knowledge about physiologically active peptides released from a parent protein source. Bioactive peptides have been defined as specific protein fragments that have a positive impact on body function or condition and may ultimately influence health (Kitts and Weiler 2003). Upon oral administration, bioactive peptides, depending on their amino acid sequence, may affect the major body systems: that is, the cardiovascular, endocrine, immune, and nervous systems (see Fig. 46.1). Below are examples of peptides which are known to exert direct or indirect physiological effects on the above systems.

The first reference to bioactive peptides in the literature was made by Mellander in 1950, who suggested that casein-derived phosphorylated peptides, caseinophosphopeptides (CPPs), enhanced vitamin D-independent bone calcification in rachitic infants (Mellander 1950). Bovine α_{s1}-, α_{s2}- and β-casein contain phosphorylated regions which

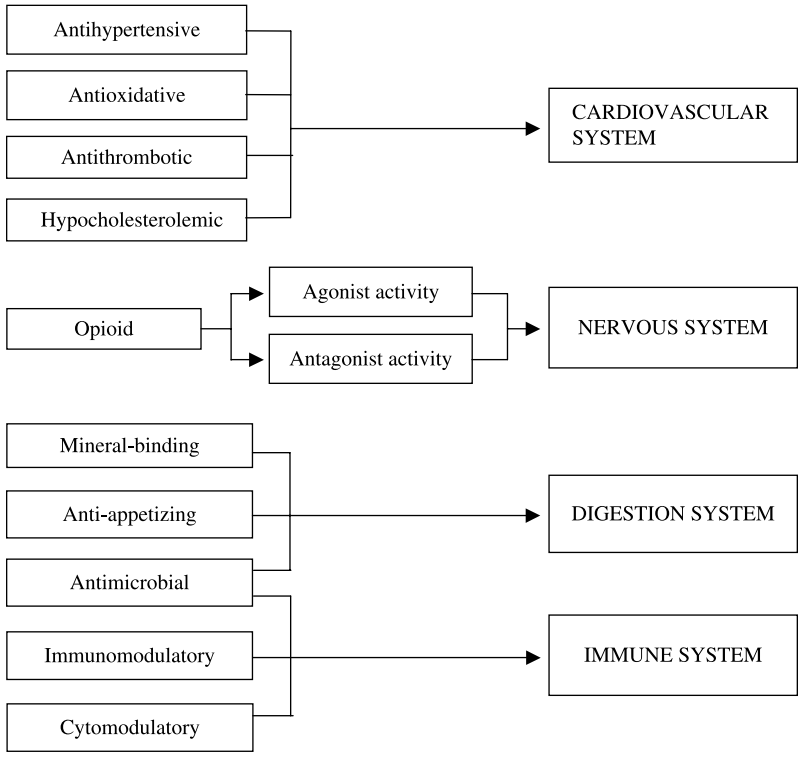

Figure 46.1 *Physiological functionality of food-derived bioactive peptides.*

can be released by digestive enzymes. Specific CPPs can form soluble organophosphate salts and lead to enhanced calcium absorption by limiting the precipitation of calcium in the distal ileum. Published data on the effect of CPPs on mineral solubility and absorption are inconsistent, however, partly due to the diversity of the experimental approaches used (FitzGerald 1998; Meisel and FitzGerald 2003). CPPs have also been shown to exert cytomodulatory effects. Cytomodulatory peptides derived from casein fractions inhibit cancer cell growth or stimulate the activity of immunocompetent cells and neonatal intestinal cells (Meisel and FitzGerald 2003).

Peptides with opioid activity have been found in milk protein and wheat gluten hydrolysates (for a review see Teschemacher 2003). Opioid peptides are opioid receptor ligands with agonistic or antagonistic activities. β-Casein opioid peptides (β-casomorphins) have been detected in the duodenal chyme of minipigs and in the human small intestine as a consequence of *in vivo* digestion of casein or milk (Meisel 1998; Meisel and FitzGerald 2000; FitzGerald and Meisel 2003). Opioid peptides may influence the gastrointestinal function in two ways: first, by affecting smooth muscles, which reduces the transit time, and second, by affecting the intestinal transport of electrolytes, which explains their anti-secretory properties. The actual physiological effects of dietary opioid peptides remain to be confirmed.

The angiotensin I-converting enzyme (ACE, peptidyldipeptide hydrolase, EC 3.4.15.1) has been associated with the renin-angiotensin system, which regulates peripheral blood pressure. Inhibition of this enzyme can exert an antihypertensive effect. ACE-inhibitory peptides have been isolated from the enzymatic digest of various food proteins and they

are, at present, the most studied group of bioactive peptides. A great number of hypotensive peptides have been identified in milk proteins and a few of these have proven clinically effective in animal model and human model studies (for reviews see Yamamoto and Takano 1999; Yamamoto and others 2003; FitzGerald and others 2004; Gobbetti and others 2004; Li and others 2004; Vermeirssen and others 2004). It was shown recently that apart from ACE inhibition, milk whey protein-derived peptides may exert antihypertensive effects through other mechanisms, such as inhibition of the release of endothelin-1 by endothelial cells (Maes and others 2004).

Peptide sequences which inhibit the aggregation of blood platelets and the binding of the human fibrinogen γ-chain to platelet surface fibrinogen receptors have been identified in caseinomacropeptide (CMP), which is split from κ-casein in milk coagulation with rennin (Fiat and others 1993). The potential physiological effects of these antithrombotic peptides have not been established, but such peptides have been detected in the plasma of newborn children after breastfeeding or ingestion of cow milk-based infant formulae (Chabance and others 1995).

Milk protein hydrolysates and peptides derived from caseins and major whey proteins can enhance immune cell functions, measured as lymphocyte proliferation, antibody synthesis and cytokine regulation (Gill and others 2000). Of special interest are casein peptides released during milk fermentation with lactic acid bacteria, as these peptides have been found to modulate the proliferation of human lymphocytes, to down-regulate the production of certain cytokines and to stimulate the phagocytic activities of macrophages (for reviews see Korhonen and Pihlanto 2003a,b,c; Matar and others 2003). The protective effect of a casein-derived immunopeptide on resistance to microbial infection by *Klebsiella pneumoniae* has been demonstrated in mice (Migliore-Samour and others 1989). Also, it has been suggested that immunomodulatory milk peptides may alleviate allergic reactions in atopic humans (Korhonen and Pihlanto 2003a).

Antimicrobial peptides have been purified from several bovine milk protein hydrolysates, but also from edible plants, fish and eggs (Clare and others 2003; Floris and others 2003; Pellegrini 2003; Gobbetti and others 2004). The most studied are the lactoferricins, derived from bovine and human lactoferrin (Kitts and Weiler 2003; Wakabayashi and others 2003). These peptides exhibit antimicrobial activity against various Gram-positive and -negative bacteria, yeasts and filamentous fungi. The disruption of normal membrane permeability is at least partly responsible for the antibacterial mechanism of lactoferricins. The antimicrobial mechanisms exerted by other active food-derived peptides have not been elucidated in detail and their physiological importance remains to be established.

Recent studies have shown that antioxidative peptides can be released from caseins, soybean and gelatine in hydrolysis by proteolytic enzymes (Korhonen and Pihlanto 2003a). Peptides derived from α_s-casein have been shown to have free radical-scavenging activity and to inhibit enzymatic and nonenzymatic lipid peroxidation (Suetsuna and others 2000; Rival and others 2001a,b). Nagaoka and others (2001) identified a novel hypocholesterolemic peptide (Ile-Ile-Ala-Glu-Lys) from the tryptic hydrolysate of β-lactoglobulin. This peptide suppressed cholesterol absorption by Caco-2 cells *in vitro* and elicited hypocholesterolemic activity *in vivo* in rats upon oral administration of the peptide solution. The mechanism of the hypocholesterolemic effect remains to be clarified.

Moreover, the α_{s1}-casein derived peptide f(91–100) has been demonstrated to possess anxiolytic-like stress-relieving properties not only in animal model studies but in human studies as well (Lefranc 2001).

Many milk protein-derived peptides have more than one functional role; for example, peptides from the sequence 60−70 of β-casein show immunostimulatory, opioid and ACE-inhibitory activities. This sequence has been defined as a strategic zone (Meisel 1998; Migliore-Samour and Jollès 1988). The sequence is protected from proteolysis because of its high hydrophobicity and the presence of proline residues. Other examples of the multifunctionality of milk-derived peptides include the α_{s1}-casein fraction 194 − 199 showing immunomodulatory and ACE-inhibitory activity, the opioid peptides α- and β-lactorphin also exhibiting ACE-inhibitory activity and the caseinophosphopeptides, which possess immunomodulatory properties (Korhonen and Pihlanto 2003a).

46.3 PRODUCTION OF BIOACTIVE PEPTIDES

Basically, biologically active peptides can be produced from precursor proteins in the following ways: (1) through enzymatic hydrolysis by digestive enzymes, (2) through fermentation of protein-rich material with proteolytic starter cultures, (3) through the action of proteolytic enzymes derived from microorganisms or plants, (4) through chemical or enzymatic synthesis, and (5) with recombinant DNA technology. In the following, examples are given of methods (1), (2), and (3), with a focus on peptides released from milk proteins. Methods (4) and (5) are currently not considered appropriate for industrial-scale production of food-grade peptides and are discussed only briefly in this review.

46.3.1 Enzymatic Hydrolysis

At present, the most common way to produce bioactive peptides is through enzymatic hydrolysis of whole protein molecules. As shown in Table 46.1, gastrointestinal enzymes, preferably pepsin and trypsin, have been used for the production of many known bioactive peptides. For example, ACE-inhibitory peptides and CPPs are most commonly produced by trypsin (see reviews Yamamoto and others 2003; FitzGerald and others 2004; Gobbetti and others 2004; Vermeirssen and others 2004). Moreover, ACE-inhibitory peptides have recently been identified in the tryptic hydrolysates of bovine α_{s2}-casein (Tauzin and others 2002) and in bovine, ovine, and caprine κ-casein macropeptides (Manso and López-Fandino 2003). Other digestive enzymes and different enzyme combinations of proteinases – including alcalase, chymotrypsin, pancreatin, pepsin, thermolysin, as well as enzymes from bacterial and fungal sources – have also been utilized to generate bioactive peptides from various proteins (see Table 46.1) (Kilara and Panyam 2003; Korhonen and Pihlanto 2003a). Recent studies have identified several antihypertensive peptides from egg white proteins hydrolyzed by pepsin, trypsin, or chymotrypsin (Miguel and others 2004) and from rapeseed proteins digested with subtilisin (Marczak and others 2003). Also, hypotensive peptides have been found in porcine skeletal muscle and corn protein hydrolysates after digestion with thermolysin (Arihara and others 2001; Nakashima and others 2002).

Recombinant DNA techniques have been employed for the production of specific peptides or their precursors in microorganisms. The sequence encoding antihypertensive peptide Arg-Pro-Leu-Lys-Pro-Trp, for example, has been introduced into the gene for soybean β-conglycin α′ subunit. This subunit was expressed in *Escherichia coli* recovered from the soluble fraction and purified by chromatography. The Arg-Pro-Leu-Lys-Pro-Trp

TABLE 46.1 Bioactive Peptides Derived from Various Animal and Plant Proteins.

Protein Source	Treatment	Peptide	Activity	Reference
Casein	Trypsin	Phe-Phe-Val-Ala-Pro	ACE inhibition	Migliore-Samour and Jollés (1988)
	Trypsin-chymotrypsin	Val-Glu-Pro-Ile-Pro-Tyr	Immunomodulation	Fiat and others (1993)
		Gly-Leu-Phe		Suetsuna and others (2000)
	Pepsin	Tyr-Phe-Tyr-Pro-Glu-Leu	Antioxidative	
β-Lactoglobulin	Trypsin	Ile-Pro-Ala-Val-Phe-Lys	Bactericidal	Pellegrini and others (2001)
		Trp-Leu-Ala-His-Lys	ACE inhibition	Pihlanto-Leppälä and others (2000)
		Ala-Leu-Pro-Met-His-Ile-Arg		Mullally and others (1997)
Egg ovalbumin	Chymotrypsin	Arg-Asp-Ala-Asp-His-Pro-Phe	Vasodilatation	Matoba and others (1999)
	Pepsin	Leu-Trp	ACE inhibition	Fujita and others (2000)
		Glu-Arg-Lys-Ile-Lys-Val-Tyr-Leu	Antihypertensive	
	Pepsin	Phe-Arg-Ala-Asp-His-Pro-Phe-Leu	Antihypertensive	Fujita and others (1995)
Egg white	Pepsin	Arg-Ala-Asp-His-Pro-Phe–Leu	ACE inhibition	Miguel and others (2004)
		Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu	ACE inhibition, Antioxidative	Dávalos and others (2004)
Porcine skeletal muscle	Thermolysin	Ile-Thr-Thr-Asp-Pro	ACE inhibition Antihypertensive	Nakashima and others (2002)
		Thr-Asp-Pro	ACE inhibition	Arihara and others (2001)
Chicken	Thermolysin	Ile-Lys-Trp	ACE inhibition	Fujita and others (2000)
		Leu-Lys-Pro	Antihypertensive	
Beef protein	Thermolysin and proteinase A	Val-Leu-Ala-Gln-Tyr-Lys	ACE inhibition	Jang and Lee (2005)
Dried bonito muscle	Thermolysin	Leu-Lys-Pro-Asp-Met	ACE inhibition Antihypertensive	Fujita and Yoshikawa (1999)
Alaska Pollack frame protein	Mackerel intestine enzyme	Leu-Pro-His-Ser-Gly-Tyr	Antioxidative	Je and others (2005)

Soybean	Proteinase S	Leu-Leu-Pro-His-His	Antioxidative	Chen and others (1995)
	Alcalase	Asp-Leu-Pro	Antihypertensive	Wu and Ding (2001, 2002)
		Asp-Gly		
	Monascus purpureus proteinase	Leu-Ala-Ile-Pro-Val-Asn-Lys-Pro	ACE inhibition	Kuba and others (2005)
		Leu-Pro-His-Phe		
		Ser-Pro-Tyr-Pro		
		Trp-Leu		
Genetically modified soybean protein	Trypsin and chymotrypsin	Arg-Pro-Leu-Lys-Pro-Trp	Antihypertensive	Matoba and others (2001)
Wheat germ	Alkaline protease	Ile-Val-Tyr	Antihypertensive	Matsui and others (1999, 2000)
Wheat gliadin	Acid protease	Ile-Ala-Pro	Antihypertensive	Motoi and Kodoma (2003)
Rice albumin	Trypsin	Gly-Tyr-Pro-Met-Tyr-Pro-Leu-Pro-Arg	Ileum contracting Immunostimulation	Takahashi and others (1996)
Spinach rubisco	Pepsin and pancreatin	Met-Arg-Trp-Arg-Asp	ACE inhibition	Yang and others (2003)
		Met-Arg-Trp	Antihypertensive	
		Leu-Arg-Ile-Pro-Val-Ala		
		Ile-Ala-Tyr-Lys-Pro-Ala-Gly		
Rapeseed	Subtilisin	Ile-Tyr	ACE inhibition	Marczak and others (2003)
		Arg-Ile-Tyr	Antihypertensive	
		Val-Trp		
		Val-Trp-Ile-Ser		

peptide was released from the recombinant-containing subunit after digestion with trypsin and chymotrypsin (Matoba and others 2001). Feeney and others (2001) reported that the construction of glutenin genes and their expression in *E. coli* is a viable method for producing peptides. In addition, Kim and others (1999) succeeded in expressing recombinant human α_{s1}-casein in *E. coli* and in purifying it. The trypsin digest of this protein contained several ACE-inhibitory peptides. Rao and others (2004) demonstrated the release of a bioactive peptide, H-Arg-Tyr-Leu-Pro-Thr-OH, in a transgenic tobacco plant, expressing a specifically designed precursor gene. Antimicrobial peptides derived from shrimp have been successfully expressed in the yeast *Sacchramoyces cerevisiae*, but the expression levels were relatively low (Desteoumieux and others 1999). Methylotrophic yeast, *Pichia pastoris*, has emerged as a powerful and inexpensive expression system for the production of high levels of functionally active recombinant proteins and several antimicrobial peptides (Li and others 2005). Despite significant advances, the synthesis of short sequences using genetic engineering methods often remains impractical, due to the low expression efficiencies obtained and difficulties encountered in the extraction and recovery of the product. Genetic engineering can, however, be used to produce enzymes with specific activity in such a manner that they are able to release the desired peptides from the precursor proteins.

46.3.2 Microbial Fermentation

Many industrially utilized dairy starter cultures are highly proteolytic. Bioactive peptides can, thus, be generated by the starter and non-starter bacteria used in the manufacture of fermented dairy products. The proteolytic system of lactic acid bacteria (LAB), such as *Lactococcus lactis*, *Lactobacillus helveticus*, and *Lactobacillus delbrueckii* ssp. *bulgaricus*, is already well characterized. This system consists of a cell wall-bound proteinase and a number of distinct intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases, and dipeptidases (Kunji and others 1996). Rapid progress has been made in recent years in elucidating the biochemical and genetic characterization of these enzymes. At least 16 peptidases responsible for the conversion of the released peptides into free amino acids have been identified and chemically characterized from LAB (Christensen and others 1999). The activities of peptidases are affected by growth conditions, making it possible to manipulate the formation of peptides to a certain extent (Williams and others 2002). Table 46.2 gives a list of experimental studies that have investigated the release of bioactive peptides upon fermentation of milk and plant materials using different live proteolytic microorganisms or proteolytic enzymes derived from such microorganisms.

The release of various bioactive peptides from milk proteins through microbial proteolysis has been reviewed in many recent articles and book chapters (Korhonen and Pihlanto-Leppälä 2001; Gobbetti and others 2002, 2004; Korhonen and Pihlanto 2003; Matar and others 2003). Most studies have reported the production of ACE-inhibitory or antihypertensive peptides, and also immunomodulatory, antioxidative, and antimicrobial peptides have been identified. *Lb. helveticus* is widely used as a dairy starter in the manufacture of traditional fermented milk products, such as Emmental cheese. Highly proteolytic *Lb. helveticus* strains capable of releasing ACE-inhibitory peptides, in particular, have been demonstrated in several studies. Nakamura and others (1995a,b) identified two ACE-inhibitory peptides (Val-Pro-Pro, Ile-Pro-Pro) in milk fermented with *Lb. helveticus.* Pihlanto-Leppälä and others (1998) studied the potential formation

TABLE 46.2 Release of Bioactive Peptides from Food Proteins by Various Microorganisms and Microbial Enzymes.

Substrate	Microorganisms Used	Precursor Protein	Peptide Sequence	Bioactivity	Reference
Milk	*Lactobacillus helveticus*, *Saccharomyces cerevisiae*	β-cn, κ-cn	Val-Pro-Pro, Ile-Pro-Pro	ACE inhibitory, antihypertensive	Takano (2002)
	Lactobacillus helveticus LBK16H	β-cn, κ-cn	Val-Pro-Pro, Ile-Pro-Pro	Attenuates the development of hypertension in SHR	Sipola and others (2001)
	Lactobacillus GG enzymes + pepsin and trypsin	β-cn, α_{s1}-cn	Tyr-Pro-Phe-Pro, Ala-Val-Pro-Tyr-Pro-Gln-Arg Thr-Thr-Met-Pro-Leu-Trp	Opioid, ACE inhibitory, immunostimulatory	Rokka and others (1997)
	Lactobacillus helveticus CPN 4	Whey proteins	Tyr-Pro	ACE inhibitory	Yamamoto and others (1999)
	Lactobacillus delbrueckii subsp. *bulgaricus* SS1 *Lactococcus lactis* subsp. *cremoris* FT4	β-cn, κ-cn	Many fragments	ACE inhibitory	Gobbetti and others (2000)
	Lactobacillus delbrueckii subsp. *bulgaricus* IFO13953	κ-cn	Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met	Antioxidative	Kudoh and others (2001)
	Lactobacillus rhamnosus +digestion with pepsin and Corolase PP	β-cn	Asp-Lys-Ile-His-Pro-Phe Tyr-Gln-Glu-Pro-Val-Leu Val-Lys-Glu-Ala-Met-Ala-Pro-Lys	ACE inhibitory, antioxidative	Hernandez-Ledesma and others (2004)
	Lactobacillus delbrueckii ssp. *bulgaricus*	β-cn	Ser-Lys-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile	ACE inhibitory	Ashar and Chand (2004)
	Str. salivarius ssp. thermophilus + *Lactococcus lactis* biovar. *diacetylactis*	β-cn	Ser-Lys-Val-Tyr-Pro	ACE inhibitory	Ashar and Chand (2004)

(*Continued*)

TABLE 46.2 *Continued.*

Substrate	Microorganisms Used	Precursor Protein	Peptide Sequence	Bioactivity	Reference
Whey	*Kluyveromyces marxianus* var. *marxianus*	β-lg	Tyr-Leu-Leu-Phe	ACE inhibitory	Belem and others (1999)
	Tritirachium album derived proteinase K	β-lg	Ile-Pro-Ala	Antihypertensive	Abubakar and others (1998)
Casein	*Lactobacillus helveticus* CP90 proteinase	β-cn	Lys-Val-Leu-Pro-Val-Pro-(Glu)	ACE inhibitory	Maeno and others (1996)
Soybean	Fermentation		His-His-Leu	ACE inhibitory, antihypertensive	Shin and others (2001)
	Bacillus natto or *Bacillus subtilis*		contain Ala, Phe and His residues	ACE inhibitory	Cho and others (2000)
Tofuyo Fermented Soybean food	Fermentation		Ile-Phe-Leu Thr-Leu	ACE-inhibitory	Kuba and others (2003)

Abbreviations: α_{s1}-cn, α_{s1}-casein; β-cn, β-casein; κ-cn, κ-casein; β-lg, β-lactoglobulin.

of ACE-inhibitory peptides from cheese whey and caseins during fermentation with various commercial dairy starters used in the manufacture of yogurt, ropy milk, and sour milk. No ACE-inhibitory activity was observed in these hydrolysates. Further digestion of the above samples with pepsin and trypsin resulted in the release of several ACE-inhibitory peptides. Gobbetti and others (2000) demonstrated the formation of ACE-inhibitory peptides with two dairy strains, *Lb. delbrueckii* ssp. *bulgaricus* and *Lc. lactis* ssp. *cremoris*, after fermentation of milk separately with each strain for 72 h. The most inhibitory fractions of the fermented milk mainly contained β-casein-derived peptides with inhibitory concentration (IC_{50}) values ranging from 8.0 to 11.2 μg/mL. Belem and others (1999) fermented cheese whey with *Kluyveromyces marxianus* var. *marxianus* and identified in the hydrolysate a sequence of β-lactorphin (Tyr-Leu-Leu-Phe), a peptide suggested to have antihypertensive properties. Yamamoto and others (1999) identified an ACE-inhibitory dipeptide (Tyr-Pro) from a yogurt-like product fermented with *Lb. helveticus* CPN 4 strain. This peptide sequence is present in all major casein fractions, and its concentration increased during fermentation, reaching a maximum concentration of 8.1 μg/mL in the product. Fuglsang and others (2003a,b) tested a total of 26 strains of wild-type LAB, mainly belonging to *Lc. lactis* and *Lb. helveticus*, for their ability to produce a milk fermentate with inhibitory activity towards ACE. All test strains produced ACE-inhibitory substances in varying amounts, and two of the strains had a high ACE inhibition and a high OPA index, which correlates well with peptide formation. The inhibitory effect of active fermentates on *in vivo* ACE activity was demonstrated in normotensive rats. More recently, Ashar and Chand (2004) identified an ACE-inhibitory peptide from milk fermented with *Lb. delbrueckii* ssp. *bulgaricus*. The peptide showed the sequence Ser-Lys-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile from β-casein with an IC_{50} value of 1.7 mg/mL. In combination with *Str. salivarius* ssp. *thermophilus* and *Lc. lactis* biovar. *diacetylactis*, a peptide structure with a sequence of Ser-Lys-Val-Tyr-Pro was obtained from β-casein with an IC_{50} value of 1.4 mg/mL. Both peptides were markedly stable to digestive enzymes, acidic and alkaline pH, as well as during storage at 5 and 10°C for four days. Furthermore, a peptide containing alanine, phenylalanine, and histidine and showing ACE-inhibitory activity has been isolated from soybeans fermented with *Bacillus natto* and chunggugjang fermented with *Bacillus subtilis.* In both studies, the optimal conditions for production were 60 h fermentation at 40°C (Cho and others 2000).

A number of studies have demonstrated that hydrolysis of milk proteins by digestive and/or microbial enzymes may produce peptides with immunomodulatory activities (Gill and others 2000; Knowles and Gill 2004). Matar and others (1996, 2001) detected immunostimulatory peptides in milk fermented with a *Lb. helveticus* strain. Sütas and others (1996a) demonstrated that digestion of casein fractions with both pepsin and trypsin produced peptides that provoked *in vitro* immunomodulatory effects on human blood lymphocytes. Peptides derived from total casein and α_{s1}-casein mainly suppressed the proliferation of lymphocytes, while those derived from β- and κ-casein primarily stimulated the proliferation rate. When the caseins were hydrolyzed by enzymes isolated from a probiotic strain of *Lactobacillus GG* var. *casei* prior to pepsin-trypsin treatment, all hydrolysate fractions were immunosuppressive and the highest activity was again found in α_{s1}-casein. The same hydrolysates also down-regulated *in vitro* the generation of interleukin-4 by lymphocytes (Sütas and others 1996b). These results suggest that LAB may modulate the immunological properties of milk proteins prior to or after oral ingestion of the product. Such modulation may be beneficial in the down-regulation of

hypersensitivity reactions to ingested proteins in patients with food protein allergies. In a clinical study, supplementation of an extensively hydrolyzed whey formula with *Lactobacillus* GG or *Bifidobacterium lactis* B12 has been shown to be more effective on eczema alleviation in infants with atopic eczema than an unsupplemented formula (Isolauri and others 2000). Further studies are needed to elucidate the potential synergistic role of probiotic bacteria and immunomodulatory milk protein peptides in the development of oral tolerance and in the management of atopic diseases.

Antioxidative peptides have been observed in milk fermented with dairy starters. Kudoh and others (2001) demonstrated that an antioxidative peptide was formed from κ-casein during *Lb. delbrueckii* ssp. *bulgaricus* fermentation. The relatively long peptide Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met showed about five times stronger antioxidative activity than the widely used synthetic antioxidant, butyl hydroxyl toluene (BHT).

In addition to live microorganisms, proteolytic enzymes isolated from LAB have been successfully employed to release bioactive peptides from food proteins. Yamamoto and others (1994) reported that casein hydrolyzed by the cell wall-associated proteinase from *Lb. helveticus* CP790 showed antihypertensive activity in spontaneously hypertensive rats (SHR). Several ACE-inhibitory and one antihypertensive peptide were isolated from the hydrolysate. Maeno and others (1996) hydrolyzed casein using the same proteinase and identified a β-casein-derived antihypertensive peptide from the hydrolysate. The antihypertensive effect of this peptide (Lys-Val-Leu-Pro-Val-Pro-Gln) was dose-dependent in SHR at a dosage level from 0.2 to 2 mg of peptide per kg body weight. The peptide did not show strong ACE-inhibitory activity as such, but a corresponding synthetic hexapeptide without Gln (Lys-Val-Leu-Pro-Val-Pro) exhibited both strong ACE-inhibitory activity and a significant antihypertensive effect in SHR as well. These results suggested that both the proline residue in the C-terminus and the amino acid sequence might be important for ACE inhibition of the hexapeptide. In a recent study, Mizuno and others (2004) measured the ACE-inhibitory activity of casein hydrolysates upon treatment with nine different commercially available proteolytic enzymes. Among these enzymes, a protease isolated from *Aspergillus oryzae* showed the highest ACE-inhibitory activity *in vitro* per peptide. The *A. oryzae* peptide also had the highest antihypertensive effect in SHR when systolic blood pressure was measured 5 h after oral administration of 32 mg/kg of various enzymatic hydrolysates. The effect was found to be dependent on the peptide dosage. These results suggested that the casein hydrolysate contained short peptides of X-Pro and X-Pro-Pro sequences. Most of the documented ACE-inhibitory peptides are usually short peptides with a proline residue at the carboxyl terminal end. Also, proline is known to be resistant to degradation by digestive enzymes and may pass from the small intestines into the blood circulation in the sequence of short peptides (Yamamoto and others 2003). This hypothesis is supported by a study by Pan and others (2004), who hydrolyzed skimmed milk with a cell-free extract of *Lb. helveticus* JCM1004 and purified the antihypertensive tripeptides Val-Pro-Pro and Ile-Pro-Pro from the hydrolysate with three runs of HPLC. The IC_{50} values of the peptides were 9.13 ± 0.21 and 5.15 ± 0.17 μM, respectively. A significant decrease ($p < 0.01$) in systolic blood pressure in SHR was measured after a single gastric intubation of Val-Pro-Pro or Ile-Pro-Pro at 8 or 4 h, respectively. Recently, Ueno and others (2004) purified and characterized an endopeptidase from *Lb. helveticus* CM4 and demonstrated that this peptidase can generate the above two antihypertensive peptides using synthetic pro-peptides as a substrate.

46.3.3 Enrichment of Bioactive Peptides

Industrial-scale production of bioactive peptides from food proteins has been limited by a lack of suitable technologies. Until now, membrane separation techniques have provided the best technology available for the enrichment of peptides with a specific molecular weight range (for a review see Korhonen and Pihlanto 2003b). Ultrafiltration has been routinely employed to enrich bioactive peptides from protein hydrolysates. Enzymatic hydrolysis can be performed through conventional batch hydrolysis or continuous hydrolysis using ultrafiltration membranes. The traditional batch method has several disadvantages, such as the relatively high cost of the enzymes and their inefficiency as compared with a continuous process.

Use of enzymatic membrane reactors for the continuous production of specific peptide sequences was introduced during the 1990s. It has already been widely applied for total conversion of food proteins of various origins to hydrolysates with improved nutritional and/or functional properties (Perea and Ugalde 1996; Martin-Orue and others 1999). Ultrafiltration membrane reactors have been shown to improve the efficiency of enzyme-catalyzed bioconversion and to increase product yields, and they can be easily scaled up. Furthermore, ultrafiltration membrane reactors yield a consistently uniform product with desired molecular mass characteristics. Continuous extraction of bioactive peptides in membrane reactors has been mainly applied to milk proteins. Bouhallab and Touzè (1995) employed this technique for the recovery of antithrombotic peptides derived from hydrolyzed CMP. Bordenave and others (1999) demonstrated that α-lactorphin could be successfully generated with continuous hydrolysis of goat whey in an ultrafiltration reactor. Gauthier and Pouliot (1996) combined enzymatic hydrolysis and ultrafiltration in order to produce emulsifying peptides from β-lg. They noted severe fouling problems with peptide–membrane interactions, especially in ultrafiltration of casein hydrolysates. A better understanding of the surface properties of the ultrafiltration membranes is therefore critical for the development of novel applications of milk protein hydrolysates. Righetti and others (1997) proposed a multicompartment enzyme reactor operating under an electric field for continuous hydrolysis of milk proteins. This technique allowed for the continuous harvesting of some biologically active peptides, such as phosphopeptides and precursors of casomorphins from the tryptic digest of β-casein.

Stepwise ultrafiltration using cut-off membranes of low molecular mass have been found useful for separating out small peptides from high molecular mass residues and remaining enzymes. Turgeon and Gauthier (1990) used a two-step ultrafiltration process and were able to produce a mixture of polypeptides and a fraction rich in small peptides with a molecular mass below 2000 Da. Pihlanto-Leppälä and others (1996) applied selective ultrafiltration membranes (30 kDa and 1 kDa) for the enrichment of the opioid peptides α-lactorphin and β-lactorphin from pepsin-hydrolyzed α-lactalbumin and from pepsin- and trypsin-hydrolyzed β-lactoglobulin, respectively. The same technique was successfully used to enrich ACE-inhibitory peptides from purified α-la and β-lg (Pihlanto-Leppälä and others 2000).

Several ion exchange chromatographic methods have been developed for the enrichment of CPPs from casein hydrolysates, but the production costs of this technique have been prohibitive for large-scale operation. Ellegård and others (1999) developed a process-scale method for the isolation of high-purity CPPs using acid precipitation, diafiltration and anion-exchange chromatography. Promising novel techniques have been developed since, based on ion exchange membrane chromatography. Recio and Visser (1999) described a method where the protein of interest was concentrated within a chromatographic

medium and hydrolyzed in situ by an appropriate enzyme. The resulting active peptides were retained on the ion exchanger, while the other peptides were washed out. Finally, the fraction containing the active peptides was eluted from chromatographic medium. With this method it was possible to isolate and enrich cationic antibacterial peptides from lactoferrin and α_{s2}-casein, as well as negatively charged phosphopeptides from β-casein. The advantage of this process is that isolation of the precursor protein is unnecessary, which significantly reduces the costs of production. Furthermore, since the enzyme used in this process can be recovered, the price of the final product can be reduced. These techniques provide new possibilities for enriching peptides with low molecular mass, and are easy to up-scale to yield gram or even kilogram quantities (Recio and others 2000).

46.4 OCCURRENCE OF BIOACTIVE PEPTIDES IN FOODS

46.4.1 Milk and Dairy Products

It is now well established that bioactive peptides can be generated during milk fermentation with the starter cultures traditionally employed by the dairy industry. As a result, peptides with various bioactivities can be found in an active form even in the final products, such as in fermented milks and cheese (for reviews see Meisel and Bockelmann 1999; Korhonen and Pihlanto-Leppälä 2001; Gobbetti and others 2002; Korhonen and Pihlanto 2003b,c, 2004; Matar and others 2003). Such traditional dairy products may, thus, under certain conditions carry specific health effects when ingested as part of the daily diet. Table 46.3 lists a number of studies which have established the occurrence of peptides in various fermented milk products. Some of these studies are described in more detail below.

A great variety of peptides are formed during cheese ripening, many of which have been shown to exert biological activities. CPPs have been found as natural constituents in fermented cheese (Roudot-Algaron and others 1994; Singh and others 1997), and secondary proteolysis during cheese ripening may lead to the formation of other bioactive peptides. ACE-inhibitory activity has been observed in many studies to be dependent on the ripening stage of the cheese. Meisel and others (1997) detected higher ACE-inhibitory activities in middle-aged Gouda cheese than in short-termed or long-termed ripened cheese. These results suggest that the concentration of active peptides in cheese increases with cheese maturation, but starts to decline when proteolysis exceeds a certain level. Accordingly, ACE-inhibitory activity was low in products having a low degree of proteolysis, such as yogurt, fresh cheese, and quark. The above findings are consistent with the results obtained by Ryhänen and others (2001), who observed that ACE-inhibitory peptides developed gradually during cheese ripening and their concentration was highest in a Gouda-type cheese at the age of 13 weeks, declining slowly thereafter. Saito and others (2000) observed ACE-inhibitory activity in several cheese varieties and measured the highest activity in Gouda cheese aged two years. In feeding experiments on SHR, the decrease in systolic blood pressure was statistically significant with four cheese varieties (Gouda, Blue, Edam, and Havarti) at 6 h after gastric intubation. Several peptides were isolated and identified from 8-month-old Gouda cheese, and two peptides derived from α_{s1}-casein f(1–9) and β-casein f(60–68), respectively, showed potent ACE-inhibitory activity. For Manchego cheese, which is prepared from ovine milk, only cheese that was at least 15 days old showed comparatively low ACE-inhibitory

TABLE 46.3 Bioactive Peptides Identified in Fermented Milk Products.

Product	Examples of identified bioactive peptides	Bioactivity	Reference
Parmigiano-Reggiano cheese	β-cn f(8-16), f(58-77), α_{s2}-cn f(83-33)	Phosphopeptides, precursor of β-casomorphin	Addeo and others, (1992)
Cheddar cheese	α_{s1}- and β-casein fragments	Several phosphopeptides	Singh and others (1997)
Italian cheeses varieties: Mozzarella, Crescenza, Italico, Gorgonzola	β-cn f(58-72)	ACE inhibitory	Smacchi and Gobbetti, (1998)
Enzyme modified cheese	β-cn f(60-66)	Opioid activity, ACE inhibitory	Haileselassie and others (1999)
Gouda cheese	α_{s1}-cn f(1-9), β-cn f(60-68)	ACE inhibitory	Saito and others (2000)
Festivo	α_{s1}-cn f(1-9), f(1-7), f(1-6)	ACE inhibitory	Ryhänen and others (2001)
Emmental cheese	α_{s1}- and β-casein fragments	Immunostimulatory several phosphopeptides, antimicrobial	Gagnaire and others (2001)
Manchego cheese	Ovine α_{s1}-, α_{s2}-, and β-casein fragments	ACE inhibitory	Gomez-Ruiz and others (2002)
Emmental cheese	Active peptides not identified	ACE inhibitory	Parrot and others (2003)
Sour milk	β-cn f(176-188)	Precursor of ACE inhibitory	Kahala and others (1993)
Sour milk	β-cn f(74-76, f(84-86) κ-cn f(108-111)	Antihypertensive	Nakamura and others (1995a)
Yoghurt	Active peptides not identified	Weak ACE-inhibitory	Meisel and others (1997)

Abbreviations: α_{s1}-cn, α_{s1}-casein; β-cn, β-casein; κ-cn, κ-casein.

activity (Gomez-Ruiz and others 2002). Furthermore, this inhibitory activity decreased during the first four months, increased when proteolysis advanced, and decreased again in 12-month-old cheese. A total of 22 peptide fragments were identified in the chromatographic fractions, corresponding to the sequences of ovine α_{s1}-, α_{s2}- and β-casein. Interestingly, peptides isolated from several Italian cheese varieties have been observed to be able to inhibit the activity of the proteolytic enzymes of LAB and ACE (Smacchi and Gobbetti 1998). Furthermore, these ACE-inhibitory peptides isolated from Italian cheeses were shown to be inhibitory to spoilage by microbial enzymes, such as thermostable proteinases from psychrotrophic *Pseudomonas fluorescens*, which causes bitterness in ultra-high temperature (UHT) treated milk, contributes to age gelation of UHT milk, and drastically reduces the shelf-life of dairy products. Active opioid peptides or casomorphins have not been found in matured Cheddar cheese, perhaps due to their degradation during the ripening process (Muehlenkamp and Warthesen 1996). On the other hand, Sabikh and Mathur (2001) detected a small quantity of β-casomorphin-3 in Edam cheese during ripening, whereas longer casomorphins were not detectable. These studies suggest that the presence of bioactive peptides which are naturally formed in cheese depends on the equilibrium between their formation and the degradation exerted by the proteolytic systems involved in the ripening process. In another study, Gagnaire and others (2001) identified a total of 91 peptides in Emmental cheese, 28 of which showed various bioactivities *in vitro*, for example, mineral-carrying, antimicrobial, antihypertensive, and immunostimulatory activities. Besides the well-known action of plasmin on β- and α_{s2}-caseins, two other proteinases seem to be involved in the hydrolysis of α_{s1}-casein in Emmental cheese: cathepsin D originating from milk and cell-envelope proteinase from thermophilic starters. Moreover, peptidases released from both starter and nonstarter LAB seem to contribute to the formation of bioactive peptides throughout the ripening period. Apart from generation during the ripening process, more bioactive peptides are likely to be formed in the gastrointestinal tract upon ingestion of a piece of cheese. This was demonstrated under *in vitro* conditions by Parrot and others (2003), who showed that the consecutive digestion of the water-soluble extract (WSE) of Emmental cheese with pepsin and trypsin, respectively, induced an increase in ACE inhibition as compared with undigested WSE. On the other hand, a 10 kDa ultrafiltered WSE lost a part of its ACE-inhibitory activity after the above digestion process. These results suggest that the generation of ACE-inhibitory peptides during digestion depends on the molecular weight of the precursor peptides present in the cheese. Hernández-Ledesma and others (2004a) evaluated the ACE-inhibitory activity of several commercial fermented milks and fresh cheeses and found that most of these products showed moderate inhibitory activity. The ACE-inhibitory activity of these commercial products remained stable or increased after simulated gastrointestinal digestion with pepsin and Corolase PP (from pig pancreas, showing mainly trypsin and chymotrypsin activities). In further studies, Hernández-Ledesma and others (2004b) evaluated the ACE-inhibitory activity of several infant formulas and found that most of these products showed moderate inhibitory activity, except for two extensively hydrolyzed milk protein-based formulas (one whey and one casein formula). A nonhydrolyzed milk protein-based formula and an extensively hydrolyzed whey formula were subjected to the same simulated gastrointestinal digestion treatment as described above. It was observed that the ACE-inhibitory activity of the nonhydrolyzed formula increased during the treatment, while no significant change was noted in the activity of the hydrolyzed whey formula after digestion. The results support the view that physiological digestion may promote the formation of bioactive

peptides from the proteins and oligopeptides present in dairy products and that at least part of the active peptides survive the digestion process.

The occurrence of various bioactive peptides in fermented milks, such as yogurt, sour milk, and "Dahi", has been reported in many studies (see Table 46.3). ACE-inhibitory, immunomodulatory, and opioid peptides, for example, have been found in yogurt and in milk fermented with a probiotic *Lb. casei* ssp. *rhamnosus* strain. In most studies, strongly proteolytic *Lb. helveticus* strains have been employed for the production of antihypertensive peptides in fermented milk products (FitzGerald and others 2004; Gobbetti and others 2004; Li and others 2004). At present, at least two fermented sour-milk products containing the ACE-inhibitory tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) have been launched commercially in Japan and Finland, respectively. The Japanese product "Calpis" is fermented with a culture containing *Lb. helveticus* and *S. cerevisiae* (Takano 1998) and the Finnish product "Evolus" contains peptides produced with *Lb. helveticus* LBK-16 H strain (Seppo and others 2002). In animal model studies, single oral administration of these products has been shown to have an antihypertensive effect in SHR (Nakamura and others 1995b; Sipola and others 2002), and "Evolus" has also been demonstrated to prevent the development of hypertension in SHR (Sipola and others 2001). As described in Chapter 5, both of these fermented drinks have proven effective in the reduction of blood pressure in mildly hypertensive human subjects.

An increasing number of ingredients containing specific bioactive peptides based on casein or whey protein hydrolysates have been launched on the market within the past few years or are currently under development by international food companies. Such peptides possess, for example, anticariogenic, antihypertensive, mineral-binding, and stress-relieving properties (Korhonen and Pihlanto 2003b).

46.4.2 Eggs

Even though eggs are a very valuable source of proteins for human nutrition, very few bioactive peptides have been described as arising from egg proteins. Hydrolyzed egg yolks have been shown to inhibit ACE action *in vitro* and to suppress the development of hypertension in SHRs after oral administration for 27 weeks (Yoshii and others 2001). Chymotryptic digestion of ovalbumin yields a vasorelaxing peptide, ovokinin (2–7) (Arg-Ala-Asp-His-Pro-Phe), which corresponds to residues 359–364 of ovalbumin (Matoba and others 1999). Much attention has been directed towards clarifying the vascular effects of ovokinin (2–7). Oral administration of this peptide has been shown to reduce systolic blood pressure in SHR, for example, but not in normotensive Wistar-Kyoto rats (WKY). On the other hand, intravenous administration of ovokinin (2–7) caused no significant changes in blood pressure except for the highest tested concentration (100 mg/kg), at which level the peptide produced a small, transient fall in blood pressure. Furthermore, ovokinin (2–7) caused an endothelium-dependent vasorelaxation in isolated mesenteric arteries in SHR – but not in WKY – mediated via nitric oxide and insensitive to B_1 and/or B_2 bradykinin receptor antagonists (Matoba and others 2001). The cardiovascular response of ovokinin is mediated by the activation of vascular B_2 receptors (Scruggs and others 2004). Ovokinin derivatives obtained by differential cleavage of ovalbumin or site-directed mutagenesis also showed hypotensive properties. For example, ovokinin Phe-Arg-Ala-Asp-His-Pro-Phe-Leu, which corresponds to residues 358–365 of ovalbumin, relaxes isolated canine mesenteric vessels (Fujita and others 1995).

Contrary to most orally active antihypertensive peptides derived from food proteins, ovokinins do not inhibit ACE (Matoba and others 2001; Yamada and others 2002). ACE-inhibitory activities were further observed in ovalbumin hydrolysates, and six peptides with IC_{50} values ranging from 0.4 to 15 μM were isolated. Five of these peptides also showed antihypertensive activities in SHR (Fujita and others 2000). Miguel and others (2004) isolated several ACE-inhibitory sequences from a peptic digest of egg white. The peptides were derived from ovalbumin and their IC_{50} values ranged from 3.2 to 435.7 μM.

Dávalos and others (2004) isolated four peptides from crude egg white hydrolyzed by pepsin which showed oxygen radical scavenging activity higher than that of Trolox. The peptides were derived from ovalbumin. The peptide Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu was also a strong ACE inhibitor and delayed the oxidation of the low-density lipoprotein lipid.

46.4.3 Meat

Relatively little is known at present about the derivation of bioactive peptides from the muscle proteins of domestic animals (i.e., meat animals). Arihara and others (2001) purified two ACE-inhibitory peptides from the thermolysin digest of porcine myosin. The sequences Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro were found in the primary structure of the myosin heavy chain. Also, six tripeptides having parts of the sequences of the myopentapeptides demonstrated activity with IC_{50} values ranging from 66.6 to >1000 μM. An ACE-inhibitory nonapeptide (Arg-Met-Leu-Gly-Gln-Thr-Pro-Thr-Lys) has been isolated from the peptic hydrolysate of porcine troponin C (Katayama and others 2003). This peptide proved to be a stronger inhibitor than the previously reported myopentapeptides and also a noncompetitive inhibitor. The nonapeptide was slowly hydrolyzed by ACE and was estimated to be a substrate-type inhibitor. The peptide showed relatively high resistance against gastrointestinal endoproteases, that is, pepsin, chymotrypsin, and trypsin. However, it was quickly hydrolyzed by aminopeptidase M and carboxypeptidase A and B, and most of the hydrolysis products showed weak ACE inhibitory activities (Katayama and others 2004). Sarcoplasmic protein extracts from beef rump were hydrolyzed with three enzymes or their paired combination, resulting in ACE-inhibitory activity. The peptide sequence was determined as Val-Leu-Ala-Gln-Tyr-Lys (Jang and Lee 2005). Fujita and others (2000) purified several ACE-inhibitory peptides from the thermolysin digest of chicken muscle. The tripeptides Ile-Lys-Trp, Leu-Lys-Pro and Leu-Ala-Pro also exhibited antihypertensive activities following intravenous administration in SHR. In addition, peptides with antioxidant activity have been obtained from porcine myofibrillar proteins with papain treatment. The Asp-Ala-Gln-Glu-Lys-Leu-Glu showed the highest antioxidant activity among the identified peptides (Saiga and others 2003).

46.4.4 Fish

Peptides inhibitory to ACE have been isolated from fish hydrolysates. Marine ACE-inhibitory peptides can be obtained, for example, with thermolysin digestion of dried bonito. Dried bonito was first hydrolyzed using several enzymes including pepsin, chymotrypsin, trypsin, and thermolysin, followed by subsequent measurement of the ACE-inhibitory activity. The results showed that the peptide fractions derived from thermolysin digestion had the highest ACE-inhibitory activity, and initially eight peptides

were isolated and identified (Yokoyama and others 1992). Fujita and others (1999, 2001) reported further *in vivo* work on ACE-inhibitory activity derived from bonito. When the thermolysin hydrolysate was ultrafiltrated, two-fold higher ACE-inhibitory and antihypertensive activity was observed in SHR after oral administration, as compared with the original digest. This hydrolysate contained a peptide (Leu-Lys-Pro-Asn-Met) which showed long-lasting and dose-dependent antihypertensive activity in SHR after oral administration. A reduction in blood pressure by 30 mmHg was recorded in SHR given an intravenous dose of 100 μg/kg of Leu-Lys-Pro-Asn-Met. In comparison, the administration of the Leu-Lys-Pro peptide at a dose of 30 μg/kg lowered systolic blood pressure by 50 mmHg. The long-term administration (e.g., seven weeks) of dried bonito-derived peptides was noted to suppress the elevation of systolic blood pressure in a dose-dependent manner. A study conducted over an eight-week period in humans, involving 30 subjects with hypertension and borderline hypertension, produced a surprising result: blood pressure was reduced by 60–66% in individuals fed a thermolysin digest of dried bonito (Fujita and Yoshikawa 1999). Other marine sources of ACE-inhibitory peptides are Indonesian dried fish hydrolyzed by pepsin (Atswan and others 1995), sea bream scales hydrolyzed by alkaline protease (Fahmi and others 2004), and yellowfin sole frame protein hydrolyzed by α-chymotrypsin (Jung and others 2005).

Sorensen and others (2004) found that peptide fractions from fish hydrolysates and autolysates inhibited the prolyl endopeptidase activity. The highest activity was found in cod, salmon, and trout samples. The prolyl endopeptidase degrades a variety of proline-containing peptides by cleaving the internal peptide bonds at the carboxyl side of proline residues. Since neuropeptides contain proline residues, any treatment that could positively modulate central neuropeptide levels would provide a promising therapeutic approach to the treatment of cognitive deficits associated with aging and/or neurodegenerative diseases.

Acid peptide fractions from a cod stomach hydrolysate have been reported to include medium-sized peptides (500–3000 Da) with immunostimulatory activity (Gildberg and others 1996). Four peptide fractions were shown to possess an affinity to simulate leukocyte super oxide anion production from Atlantic salmon.

Carnosine, a dipeptide composed of β-alanine and L-histidine, is found in high concentrations in muscle cells including fish. Carnosine has been reported to exhibit extensive antioxidative and hydrogen buffering properties (Chan and Decker 1994). Nagasawa and others (2001) demonstrated the antioxidant activity of carnosine both *in vitro* and *in vivo*. Carnosine was found to inhibit lipid peroxidation and oxidative modification of protein in rat muscle tissue exposed to a hydroxyl radical generated from Fenton radicals. The hydrolyzed gelatin extracted from Alaska pollack skin showed antioxidative activity. Two peptides, composed of 13 and 16 amino acid residues, were isolated from the hydrolysate. The longer peptide protected rat liver cells from oxidant injury by t-BHT (Kim and others 2001). Moreover, Je and others (2005) isolated the antioxidative peptide Leu-Pro-His-Ser-Gly-Tyr from Alaska pollack frame protein hydrolysate.

Various fish sources have yielded antimicrobial peptides. Liver tissue from Atlantic salmon contains an antimicrobial peptide with a molecular mass of 20,734 Da. This purified peptide has been identified as a histone protein and is termed histone H1 (Richards and others 2001). Histone proteins are normally found in chromatin and are involved in the structural organization of DNA. Three antibacterial basic polypeptides have been isolated from acetic acid extracts of Channel catfish (*Ictalurus punctatus*) skin (Robinette and others 1998). Catfish (*Parasilurus asotus*) also contains a strong antimicrobial peptide termed parasin I, consisting of 19 amino acids that include three arginine and five

lysine residues and have a molecular mass of 2000 Da (Park and others 1998). Parasin I showed strong antimicrobial activity towards both Gram-negative and Gram-positive bacteria as well as fungi without any hemolytic activity. The peptide forms a β-strand in both a hydrophilic and a hydrophobic environment, instead of the more common linear amphipathic α-helix structure found in many other antimicrobial peptides. It has been suggested that protease digestion of histone H2A may yield a potent antimicrobial peptide, which is supported by the fact that other antimicrobial peptides, such as lactoferricin generated by pepsin digestion of bovine and human lactoferrin, also exhibit greater antimicrobial activity than the native lactoferrin.

46.4.5 Plants

Peptides with ACE- and PEP-inhibitory, immunomodulating, antimicrobial, antioxidative, and opioid activities have been isolated from food plants. Gibbs and others (2004) detected several bioactive peptides in soy-fermented foods digested with proteolytic enzymes. The activities ranged from ACE-inhibitory and antithrombotic to surface-active and antioxidative properties. Peptides inhibitory to ACE have been isolated from a tryptic isolate of zein (maize), hordein (barley) and gluten (wheat) (Gobbetti and others 1997). Three strong ACE inhibitors isolated after thermolysin hydrolysis of α-zein and characterized as Leu-Arg-Pro, Leu-Ser-Pro, and Leu-Gln-Pro contained a common C-terminal proline residue. IC_{50}-values denoting the potency to inhibit ACE *in vitro* for all these peptides were found to be very low. However, it is noteworthy that oral administration of these peptides to SHR produced a significant decrease in systolic blood pressure compared with the control at 6 h after administration (Matsumura and others 1993). ACE-inhibitory peptides prepared from soy protein hydrolyzed by alcalase were stable to gastrointestinal protease *in vitro* and also active *in vivo* in SHR (Wu and Ding 2001). Kuba and others (2004) hydrolyzed the soybean proteins β-conglycinin and glycinin by acid proteinase from *Monascus purpureus*. Four ACE-inhibitory peptides were isolated from the hydrolysates. The activities of the peptides Leu-Pro-His-Phe and Trp-Leu were preserved after digestion *in vitro*. Moreover, the inhibitory activity of Leu-Ala-Ile-Pro-Val-Asn-Pro markedly decreased whereas that of Ser-Pro-Tyr-Pro markedly increased after digestive treatment. Yang and others (2003) isolated four ACE-inhibitory peptides from a pepsin-pancreatin digest of spinach Rubisco. The peptides Met-Arg-Trp and Met-Arg-Trp-Asp showed an antihypertensive effect after oral administration at a dose of 20 and 30 mg/kg. The maximal decrease was observed 2 or 4 h after administration. The peptide Ile-AlaTyr-Lys-Pro-Ala-Gly exerted antihypertensive activity after oral administration at a dose of 100 mg/kg. Four potent ACE inhibitory peptides (Ile-Tyr, Arg-Ile-Tyr, Val-Trp-Val, and-Trp-Ile-Ser) were isolated from a subtilisin digest of rapeseed protein (Marczak and others 2003). The peptides were relatively stable to digestion with the enzymes present in the digestive tract. All isolated peptides and the subtilisin digest of rapeseed protein lowered blood pressure in SHR following oral administration. The hydrolysate significantly lowered blood pressure even at a single dosage of 0.15 g/kg. Moreover, sunflower seed proteins were recently found to be a potential source of ACE-inhibitory peptides when hydrolyzed by pepsin and pancreatin (Megias and others 2004).

Wheat gluten is a well-known source of opioid peptides among food proteins (Fukudome and Yoshikawa 1993). A peptide named oryzatensin (Gly-Tyr-Pro-Met-Tyr-Pro-Leu-Pro-Arg) with immunomodulatory activity causing smooth muscle contraction has been obtained from a rice tryptic hydrolysate. Shorter, C-terminal fragments of

oryzatensin demonstrating similar activity were also reported. Phagocytosis by leukocytes from human blood was induced *in vitro* by oryzatensin and the production of superoxide anions in leukocytes was also stimulated (Takahashi and others 1996). Six peptide fragments with antioxidant activity were isolated from digests of the soybean protein β-conglycinin (Chen and others 1995, 1996). The Leu-Leu-Pro-His-His domain played a major role in the noted antioxidative activity of this peptide. The results indicated that the Pro-His-His sequence exerted the greatest antioxidative activity among all tested peptides and had synergistic effects with nonpeptide antioxidants, for example, tocopherol, butylated hydroxyanisole, and butylated hydroxytoluene.

Many plants produce antimicrobial peptides which have a role in the defence of the plant against infection or natural invaders. A class of small, basic peptides, referred to as thionins, have been found in seeds of wheat, barley, rye, and oats (Garcia-Olmedo and others 1998). Thionins are cysteine- and lysine-rich polypeptides that contain 43–46 amino acid residues and have considerable homology among different species. Futhermore, they exhibit antimicrobial activity against plant pathogenic fungi and bacteria *in vitro*. Zhang and Lewis (1997) isolated two antimicrobial peptides, fabatins, by acid extraction from the broad bean *Vicia faba.* Fabatins have an overall positive charge and contain 47 amino acid residues and eight cysteine residues that probably derive from four disulfide bridges characteristic of thionins. Fabatins were found to exhibit activity against Gram-positive and Gram-negative bacteria, but were inactive against the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Macrocyclic cysteine-knot peptides containing 29–31 amino acid residues have been isolated from coffee plants. The macrocyclic peptides were active against Gram-positive and -negative bacteria and against fungi. Furthermore, they were cytotoxic and lysed human red cells. The potent antimicrobial activity of macrocyclic peptides is salt-dependent and their initial interactions with microbial cell surfaces may be electrostatic (Tam and others 1999).

46.5 BIOLOGICAL EFFECTS OF BIOACTIVE PEPTIDES

The potential beneficial biological effects of food protein-derived peptides have been a subject of growing commercial interest in the context of health-promoting functional foods. So far, milk protein-derived hypotensive, mineral-binding, and anticariogenic peptides have been most studied for their physiological effects in animal models and human subjects. The major reason for screening hypotensive peptides has been their ability to inhibit ACE activity, but a few studies also suggest that the hypotensive effect of peptides can be mediated by other mechanisms as well, for example, through the vasodilatory action of binding to opiate receptors or modulation of endothelin-1 release by endothelial cells (Nurminen and others 2000; Maes and others 2004). To exert an antihypertensive effect after oral ingestion, active peptides must be absorbed in an intact form from the intestine and further be resistant to degradation by plasma peptidases in order to reach the target sites. In fact, it has been demonstrated that small ACE-inhibitory peptides can be absorbed intact through the intestine by paracellular and transcellular routes, but the potency of bioactivity after absorption is inversely correlated to chain length. It is also known that proline-containing peptides are generally resistant to degradation by digestive enzymes. Many animal studies, reviewed by Li and others (2004), have shown a dose-dependent hypotensive effect in SHR after single oral administration of small di- and tripeptides. Masuda and others (1996) detected two ACE-inhibitory

tripeptides (Val-Pro-Pro and Ile-Pro-Pro) in the abdominal aorta of SHR after oral administration of sour milk containing these tripeptides. On the other hand, some peptides with weak ACE-inhibitory activities *in vitro* show strong antihypertensive activities after oral administration. One reason for this phenomenon may be that such weak peptides are converted into stronger ones through degradation by gastrointestinal proteases. These smaller peptides can thereafter be absorbed intact into the blood circulation and exhibit antihypertensive effects (for a review see Li and others 2004).

A number of *in vivo* studies with SHR and hypertensive human volunteers have reported significant blood pressure-reducing effects after consumption of specific milk protein hydrolysates and fermented dairy products (see reviews by FitzGerald and others 2004; Li and others 2004; Vermeirssen and others 2004). Table 46.4 lists human clinical studies carried out using these products. In a placebo-controlled trial with mildly hypertensive subjects a significant reduction in blood pressure was recorded after daily ingestion for four weeks of 95 mL of "Calpis" sour milk containing the potent ACE-inhibitory peptides Val-Pro-Pro and Ile-Pro-Pro. It is noteworthy that the ingested dose of these peptides was small, about 2.6 mg per day. Blood pressure was reverted gradually to pretrial level after the intervention period ceased. No major changes in blood pressure were observed in the placebo group (Hata and others 1996; Yamamoto and others 2003). These results were supported by a recent double-blind randomized controlled study (Mizushima and others 2004) in which the effect of 'Calpis' was assessed in borderline hypertensive men upon oral administration of 160 g of the product for four weeks. Systolic blood pressure in the test group decreased significantly after two and four weeks of ingestion of "Calpis." No significant change in blood pressure was observed in the placebo group, which was administered unfermented acidified milk. Similar results were obtained with the "Evolus" product in two double-blind, placebo-controlled studies with mildly hypertensive subjects who ingested 150 mL of the product daily. "Evolus" was found to decrease both systolic and diastolic blood pressure during the eight-week and 21-week treatment periods, respectively. No such influence was reported in subjects with normal blood pressure (Seppo and others 2002, 2003). Another recent placebo-controlled study (Ashar and Chand 2004) tested the effect of "Dahi" fermented milk containing the ACE-inhibitory peptide Ser-Lys-Val-Tyr-Pro (SKVYP) on hypertensive subjects. The product was produced by fermentation of milk with *Lb. delbrueckii* ssp. *bulgaricus*, *Str. salivarius* ssp. *thermophilus*, and *Lc. Lactis* biovar. *diacetylactis.* The subjects received either 100 mL of the test product or the placebo product for four weeks. In the test group, a significant decline in systolic blood pressure was recorded after two and four weeks from the start-up of the trial. No significant change in blood pressure was noticed in the placebo group during the intervention period. The placebo product was prepared using the same starters as the test product, but these strains did not produce the above ACE-inhibitory peptide.

Many animal and human studies have reported the presence of caseinphosphopeptides (CPP) *in vivo* following ingestion of milk, fermented dairy products, casein, and crude CPP preparations (Meisel and FitzGerald 2003). The stomach and intestinal contents of adult humans fed milk or yogurt were found to contain CPPs (Chabance and others 1998). CPPs have been detected in the distal small ileum (ileum) of humans administered milk or crude CPP preparations orally (Meisel and others 2003). Since CPPs can bind and solubilize minerals, they have been considered physiologically beneficial in the prevention of osteoporosis, dental caries, hypertension, and anemia. Conflicting results have been obtained in animal studies which have assessed the potential of CPPs to enhance

TABLE 46.4 ***In Vivo*** **Human Studies on Bioactive Peptides.**

Product Administered	Peptide Precursor/ Peptides Identified	Effect Observed	Reference
Rice-based cereal gruel + CPP	Caseinphospho peptides (CPP)	Improvement of calcium and zinc absorption	Hansen and others (1996)
Tryptic casein hydrolysate	α_{s1}-casein	Reduction of blood pressure	Sekiya and others (1992)
Sour milk	β-casein, κ-casein Val-Pro-Pro/ Ile-Pro-Pro	Reduction of blood pressure	Hata and others (1996)
Sour milk	Val-Pro-Pro/Ile-Pro-Pro	Reduction of blood pressure	Seppo and others (2003)
Sour milk	Val-Pro-Pro/Ile-Pro-Pro	Reduction of blood pressure	Mizushima and others (2004)
Sour milk (Dahi)	β-casein Ser-Lys-Val-Tyr-Pro	Reduction of blood pressure	Ashar and Chand (2004)

Abbreviations: α_{s1}-cn, α_{s1}-casein; β-cn, β-casein; κ-cn, κ-casein.

mineral (primarily calcium) bioavailability. In general, animal studies with the use of tracers have revealed a positive effect of CPPs on calcium absorption, whereas most of the balance studies have failed to find any effect of CPP addition (FitzGerald 1998). In human studies, increased calcium and zinc absorption has been demonstrated in adults administered a rice-based infant gruel. This beneficial effect was abolished when the volunteers were fed cereal-based meals containing phytate (Hansen and others 1996). More recently, Narva and others (2004) showed that *Lb. helveticus* fermented whey and the tripeptides Val-Pro-Pro and Ile-Pro-Pro stimulated the proliferation of osteoblasts *in vitro*, whereas sour-milk whey and calcium had no effect. The fermented whey contained 26 mg/L of these peptides. No significant effects on osteoclast formation were observed *in vitro* with any of the studied products. More cell culture and human studies are necessary to demonstrate the potential of CPPs and other peptides to enhance dietary mineral bioavailability and to modulate bone formation. Another interesting property associated with CPPs is their potential to enhance mucosal immunity. This idea is supported by a study (Otani and others 2000) which showed that oral administration of a commercial CPP preparation enhanced the intestinal IgA levels of piglets.

The anticariogenic effect of CPPs has been well documented in both human and animal studies (Meisel 2001). CPPs can have an anticariogenic effect by promoting recalcification of tooth enamel, whereas glycomacropeptide (GMP) derived from κ-casein seems to contribute to the anticaries effect by inhibiting the adhesion and growth of plaque-forming bacteria on oral mucosa (Brody 2000; Malkoski and others 2001). Various dental care products containing CPPs and/or GMP have been launched on the market in some countries.

46.6 FUTURE APPLICATIONS OF BIOACTIVE PEPTIDES

The occurrence of biologically active peptide sequences in dietary proteins is now well established. A few peptides have proven effective in the provision of specific physiological activities *in vivo* upon oral administration. The optimal exploitation of bioactive peptides for human nutrition and health poses an exciting scientific and technological challenge, while at the same time offering potential for commercially successful applications. Bioactive peptides can be incorporated in the form of ingredients in functional and novel foods, dietary supplements, and even pharmaceuticals with the purpose of delivering specific health benefits. Such tailored dietary formulations are currently being developed worldwide to optimize health through nutrition. This approach has been taken initially at target group level but will ultimately address individuals. To this end, bioactive peptides offer an excellent basis for the novel concept of "personalized nutrition." Many scientific, technological and regulatory issues must, however, be resolved before these substances can be optimally harnessed to this end.

Firstly, there is a need to develop novel technologies, such as chromatographic and membrane separation techniques, to enrich active peptide fractions from the hydrolysates of various food proteins (Korhonen 2002). In addition to enzymatic hydrolysis, microbial fermentation provides a natural technology applicable for the production of bioactive peptides either from animal or plant proteins. The potential of this approach is already well demonstrated by the presence of bioactive peptides in fermented dairy and soybean products. Production of bioactive peptides from protein-rich raw materials may be scaled up to industrial level using controlled fermentation in bioreactors with known LAB. In

future, the commercial production of specific peptide sequences is likely to employ recombined enzyme technology and specific production strains or alternatively make use of peptidases isolated from suitable microorganisms.

Secondly, it is important to study the technological properties of active peptide fractions and to develop model foods that contain these peptides and retain their activity for a guaranteed period. It is recognized that, due to their lower molecular weight, peptides can be more reactive than proteins, and the peptides present in the food matrix may react with other food components. The interaction of peptides with carbohydrates and lipids, as well as the influence of the processing conditions (particularly heating) on peptide activity and bioavailability, should also be addressed (Korhonen and others 1998). In this respect, the possible formation of toxic, allergenic, or carcinogenic substances warrants intensive research. In fact, modern analytical methods need to be developed to study the safety of foodstuffs containing biologically active peptides.

Thirdly, molecular studies are needed to assess the mechanisms by which bioactive peptides exert their activities. This research area is currently considered highly challenging, as the majority of the known bioactive peptides are not absorbed from the gastrointestinal tract into the blood circulation. Their effect is, therefore, likely to be mediated directly in the gut lumen or through receptors on the intestinal cell wall. In this respect, the target function of the concerned peptide is of utmost importance. It is anticipated that such targets will be related to various lifestyle-related disease groups, such as cardiovascular diseases, cancers, diabetes, osteoporosis, stress, and obesity. Bioactive peptides derived from dietary proteins offer a promising approach to prevent, control and even treat such disease conditions through a controlled diet.

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47

Lipid-Soluble Vitamins: Nutritional and Functional Aspects

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47.1 Introduction 40
47.1.1 Functional Foods: a New Concept in Nutrition 40
47.1.2 Nutritional and Functional Properties of the Vitamins 40
47.2 Vitamin A 42
47.2.1 Nutritional Properties of Vitamin A 43
47.2.2 Functional Properties of Vitamin A 44
47.3 Vitamin E 45
47.3.1 Nutritional Properties of Vitamin E 45
47.3.2 Functional Properties of Vitamin E 46
47.4 Vitamin D 47
47.4.1 Nutritional Properties of Vitamin D 47
47.4.2 Functional Properties of Vitamin D 48
47.5 Vitamin K 49
47.5.1 Nutritional Properties of Vitamin K 49
47.5.2 Functional Properties of Vitamin K 50
References 51

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

47.1 INTRODUCTION

47.1.1 Functional Foods: a New Concept in Nutrition

There is no doubt that our view of foods has changed enormously throughout history, and this has driven the development of technology applied to the processing of products that we consume daily along with it. But it is in the last few years that the change in dietary concepts has been most significant, revolutionizing the food industry and food science. In the past, consumers' main concerns were the risks of the presence of contaminants and the deficiencies in processing that could affect their health. These concerns now have changed to the perceived benefits of nutrients ingested in an appropriate manner and provided for healthy development and maintenance. Food was once seen as carriers of substances potentially harmful to our health, and some epidemiological and diet studies recommended the reduction or avoidance of the consumption of certain ingredients in foods. It left behind a preventive and restrictive tendency that changed the type of food and its production. However, scientific advances changed this tendency and have reached a different approximation from the diet-health binomial. Currently, our diet composition is regulated by the inclusion of positive components and the correct dosage of others, which if consumed in excess are not beneficial, instead of promoting their simple exclusion. The discovery of the positive effects on health by minority components in food (more commonly found in fruit and vegetables) brought about the change in the previously mentioned tendency, and when the positive view was extended to macronutrients, it gave rise to the concept of functional food.

A generally accepted definition of functional food would be any food or ingredient that has a positive impact on an individual's health, physical performance, or state of mind, in addition to its nutritive value (Goldberg 1994). To distinguish functional foods from nutraceuticals, it should be kept in mind that the former are foods of similar composition presented to those normally consuming them and should be included in the normal daily diet.

The market for functional food is growing at a steady rate (20% annually). The current dietary products included in this type of food are present in categories such as milk products, drinks, cereals, and meat. These foods include any or some ingredients which provide this functionality such as, for example, fiber, oligosaccharides, amino acids, peptides and proteins, isoprenoids and vitamins, minerals, polyunsaturated fatty acids, antioxidants, and phytochemicals. Despite the relevance of the functional aspect of these components, it is not easy to distinguish them from the nutritional ones, which are also present. For some of these ingredients, it is this last aspect which characterizes and defines them in a more classical way, as in the case of the vitamins.

47.1.2 Nutritional and Functional Properties of the Vitamins

The existence of specific essential components for adequate nutrition was sensed throughout history. Many observations have been described relating to the absence or deficiency of some elements in the diet (fruit, vegetables, meat, and fish) with the development of certain diseases. When the diet included, or was fortified with these elements, these diseases were not present or remitted in the population. However, it is not until the beginning of the 20th century, when specific ingredients, which enabled a proper development

and healthy state of the body, began to be identified. These investigations had the same foundations as early observations with the discovery of the vitamins:

- Absence or presence of a diet component;
- Effect on human health;
- Isolation, identification, and extraction of the responsible ingredient.

This leads to the discovery of the vitamins. The importance of the vitamins in human nutrition and health, along with the deficiencies suffered by certain population groups (and they still suffer today), brought about their cost-effective production. They were first produced by extraction from natural sources and later by synthesis. This reduced the price considerably and enabled them to be used on a large scale in the food industry. This revolutionized the market, which is inundated with foods fortified with vitamins and multivitamin preparations. The existence of these types of dietary product makes it easier for the population to complement the quantity of vitamins ingested through the normal daily diet to reach an optimal vitamin status. This status is achieved by ingesting the recommended daily amount (RDA) set by the public health authorities for each vitamin, whose objective is to avoid the manifestation of those diseases caused by the absence or deficiency of these nutrients.

More specifically, and according to the Food and Nutrition Board, the RDAs of vitamins are defined as the level of ingestion that, based on scientific knowledge, are adequate to cover the nutritional needs of, practically, all healthy individuals. A level which includes a minimum requirement for a maximum protection, is derived from each function dependent on the vitamin, as well as a minimum requirement for an adequate reserve of these nutrients.

Bearing in mind the definition of functional food and the common use of vitamins, these appear to be standardized in other types of foods more than in functional ones. In this way, they are common ingredients in food categories such as dietary supplements, medicinal foods, fortified foods, and special dietary foods (Kwak and Jukes 2001). How can the concept of functionality be defined in the vitamins? Only from the state of health reached with RDA and the objectives achieved by it, is when we can begin to distinguish and characterize the functionality of the vitamins. For this reason it is appropriate to consider two important points:

1. Only deficient and toxic levels of vitamins have negative clinical manifestations. However, the effect on health produced by levels in between those is unknown.
2. Their positive implication in the prevention of diseases that are not due to their absence or deficiency, an implication which appears when amounts greater than those recommended are ingested.

Both points highlight the current discrepancy between the RDA – defined to achieve an optimal nutritional status to avoid the diseases caused by their deficit – and the amount with which optimal functional status is acquired, a situation that is illustrated in Figure 47.1. The quantity of vitamin D adequate to prevent rickets is known but the quantity required for the prevention of other degenerative processes and chronic diseases is unknown. Within this discrepancy is where the functional character of the vitamins and their additional biological actions should be established, and supported by the scientific evidence which is continually being provided. Their role as antioxidants, immune

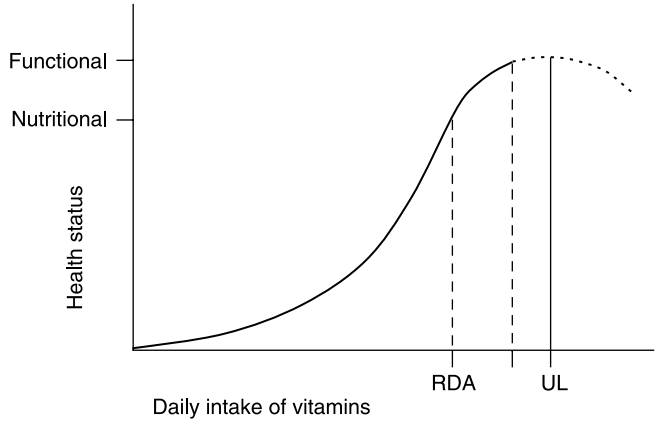


Figure 47.1 *Possible discrepancy between the RDA of vitamins and the amount with which optimal functional status is acquired, and their effects in health status of the individual.*

system stimulators, intercellular communication enhancers, and in the reduction in the risk of diseases, which occur with advanced age, determines the functionality of the vitamins (Walter and others 2000).

This area of investigation has been fairly active in the last few years, and has been given a boost by the success of functional foods. Apart from the commercial aspects, the scientific interest is seen in the determination of additional functions that the vitamins will carry out, by analyzing the mechanisms of action, the involvement of their metabolites, and the establishment of new recommended levels of ingestion. If the tendency is to increase the daily dose it, places special emphasis on the toxic effects they can produce (Walter 2001).

The present chapter deals with the most relevant nutritional and functional properties of the lipid soluble vitamins, including in each case a short introduction describing the structural characteristics of each vitamin, which make possible their interaction with other biomolecules and the development of their biological functions.

47.2 VITAMIN A

Since McCollum and Davis adopted the term "fat soluble A" in 1913, the knowledge of vitamin A has advanced enormously. Initially, discoveries such as the conversion of β-carotene into vitamin A in hepatic tissue and deficiencies of this vitamin in specific population groups were notable. In 1930, the structure of this compound was determined and that of its most active precursor (β-carotene). A decade later, Isler established a process for its synthesis from a precursor (β-ionone), making the production of this vitamin significantly cheaper. In the middle of the 20th century, many of the concepts and metabolic pathways of vitamin A were defined, which are still valid today (Olson 2001).

The group of substances known as vitamin A have a structure differentiated in two zones, a β ring and a chain with nine carbon atoms with four alternated double bonds and two side-chain methyl groups. The final carbon atom presents oxygen functions, from which a varied group of compounds is denoted, being retinol, retinal, and retinoic acid the most representative. Vitamin A occurs in its esterified form, and it is in

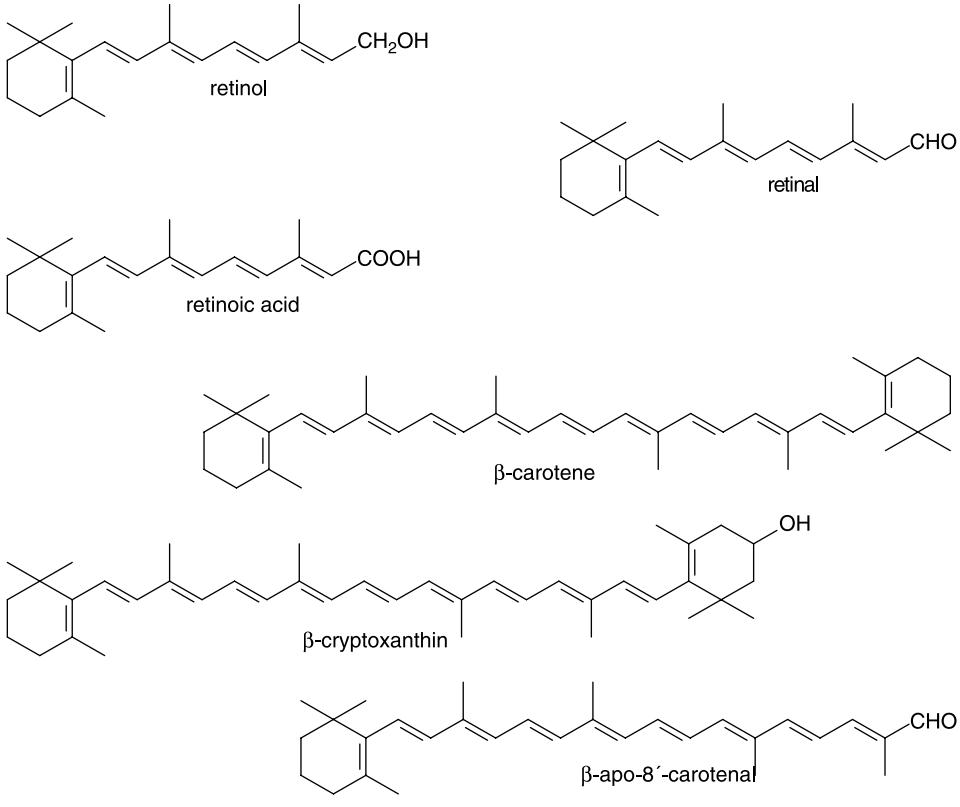


Figure 47.2 *Structure of retinol and its derivatives, and main carotenoids with provitamin A activity.*

this state that it is normally marketed (acetate esters of retinyl and retinyl palmitate). The precursors of vitamin A, the carotenoids, have a wide diversity of structures (in nature, more than 600 carotenoids are known) but only 10% show provitamin A activity in complying with the structural requirements for this (a β ring without oxygen functions). In Figure 47.2, the structures of the most representative retinoids and carotenoids with provitamin A activity are shown.

47.2.1 Nutritional Properties of Vitamin A

The dietary requirements of vitamin A are usually supplied by retinol (mainly in esterified form) and the carotenoids with provitamin A activity. The most common foods in which this vitamin is obtained are milk products, liver, and fish oils. The carotenoids present in carrots, green vegetables such as spinach, fruits like sweet peppers, mango, and papaya, and vegetable oils such as that from palm generally contain one or several precursors with provitamin A activity (Booth and others 1992).

The role of vitamin A is seen in many different metabolic processes and is involved in growth and development, immune function, reproduction, maintenance of the integrity of epithelial cells, and the correct functioning of the visual system. The RDA for adults is reduced from the recommended values of 1000/800 μg retinol/day (males/females) (National Research Council 1989), to currently 900/700 μg retinol/day. As was

mentioned in the introduction, this amount prevents the appearance of specific symptoms of deficiency such as xerophthalmia and irreversible blindness and other nonspecific ones such as lack of development and growth, and increasing risk of anemia and infectious diseases. Even today, vitamin A deficiency is a problem on a world scale. WHO estimates that annually, three million children develop some type of xerophthalmia and another 250 million are deficient in this vitamin. It is no surprise that plans are continually being put into operation to avoid these problems in the developing countries (Gibson and others 2000); the most well known amongst them is the development of a type of rice rich in β-carotene known as "golden rice." On occasions the development and production of functional foods in western countries copy these strategies, which increase the bioavailability of this and other micronutrients.

47.2.2 Functional Properties of Vitamin A

Apart from the nutritional properties of the group of substances which encompasses vitamin A (retinoids and carotenoids with provitamin A activity), they carry out other biological actions important to human health. The retinoids are loosely classified as antiproliferative agents. Although they are also proposed as antioxidants, this action appears to be associated more with the antioxidant capacity of the provitamin A, β-carotene, and the synergy effect of this compound with the antioxidant vitamins E and C (Combs 1998).

Regarding the decrease in the risk of developing degenerative diseases, such as cancer, it is stated in the control of cellular differentiation and proliferation, and the induction of apoptosis, actions mediated by vitamin A (Harris 1990; Prowse and others 2002). These processes are of special importance in those tissues which are exposed to an increased cycle of cellular loss and renewal such as the digestive tract. In this case, the induction of apoptosis controls the number of damaged, pre- and neoplasic cells, an effect which the retinoids induce by means of the activation of calcium-dependent transglutaminases (Fesus and others 1996).

Another two important processes in the functionality of the retinoids are their roles as transcriptional regulators (Murakami and others 2002) and stimulators of intercellular communication (GJC). This activity is deficient in many cancerigenic cells and the stimulation or restoration of GJC should produce a decrease in their proliferation. Since an effective function of the immune system is based on intercellular communication, the provision of GJC by the retinoids collaterally boosts immune activity.

Like all lipid soluble vitamins, excessive storage of vitamin A, mainly in the liver, generates toxicity. The Food and Nutrition Board stipulates a maximum daily dose of 3000 μg to avoid the appearance of secondary effects derived from the toxicity of this vitamin, such as headaches and vomiting, desquamation of the skin and hepatic damage. More recently effects harmful to bone health have been reported. The publications regarding this effect suggested a correlation between chronic ingestion of vitamin A in amounts greater than the RDA and hip fractures (Promislow and others 2002; Penniston and Tanumihardjo 2003).

Epidemiological studies on the possible harmful effect of the carotenoids with provitamin A activity, specifically β-carotene, have generated more controversy. The negative effect that supplementation with β-carotene produced in risk groups (such as heavy smokers), has highlighted that the metabolism of this provitamin A produces oxidized compounds similar in structure to that of the retinoids, interfering in the signaling pathways, and that an excess of these components can induce the activity of cytochrome

P450 enzymes (Wang and Russell 1999). This situation produces an increase in the catabolism of retinoids and with this a reduction in the control of cellular differentiation and proliferation. The individualization and excess ingestion of these micronutrients are factors which trigger their negative effects on human health.

47.3 VITAMIN E

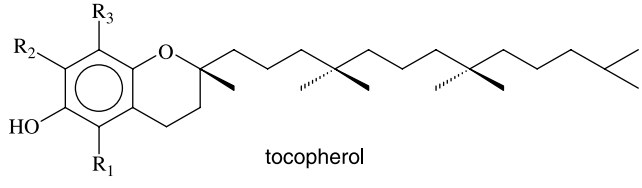
Although the discovery of vitamin E occurred in 1922, when Evans and Bishop determined the essentiality of a lipophilic substance in rodent reproduction, it was not until the 1960s that the importance of this vitamin in humans was recognized with the association of its deficiency with the development of anemia in premature babies (Machlin 1991). The term vitamin E encompasses a group of lipophilic substances (four tocopherols and four tocotrienols) whose main activity is as an antioxidant against free radicals.

The structure of these compounds has a 6-chromanol ring (head) and a phytyl side chain (tail). The position and number of methyl groups differentiate each component, whilst the presence or absence of double bonds in the tail characterizes the tocotrienols and tocopherols, respectively (Fig. 47.3). Each tocopherol/tocotrienol has a different antioxidant ability depending on its structural characteristics; α-tocopherol (the most abundant in nature) shows the greatest capacity (Foote 1979; Fukuzawa and Gebicki 1992).

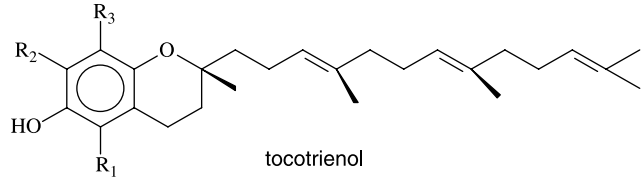
47.3.1 Nutritional Properties of Vitamin E

Vitamin E, due to its great capacity and synergy, represents the nonenzymatic antioxidant system, which along with the enzymatic antioxidant group, controls oxidative stress, its

Substituent groups	Tocopherol	Tocotrienol
$R_1=R_2=R_3=CH_3$	α	α
$R_1=R_3=CH_3$ $R_2=H$	β	β
$R_1=H$ $R_2=R_3=CH_3$	γ	γ
$R_1=R_2=H$ $R_3=CH_3$	δ	δ



tocopherol



tocotrienol

Figure 47.3 *Structure of vitamin E (tocopherols and tocotrienols).*

adverse effects and the reactive intermediates that they produce. A severe deficiency of vitamin E produces anemia and neuromuscular abnormalities. Except for those individuals with a defect in the gene that regulates the α-tocopherol transfer protein, vitamin E deficiency is not normal in humans and with the supply coming from the diet it reaches an adequate optimum status (Ouahchi and others 1995; Traber and Sies 1996). Vegetable oils, such as soya and corn, margarines, enriched cereals, tomato, and whole milk products, eggs, and meat are common sources of vitamin E. The Food and Nutrition Board sets a RDA of 15 mg/day, assigning antioxidant activity as the main function of this vitamin. As it will be mentioned in the following section, the metabolic functions of this group of substances are not wholly defined but it appears that it goes beyond their activity as antioxidants.

The implication of free radicals in the genesis of diseases, such as arteriosclerosis, cataracts, certain types of cancers, neurodegenerative disorders, and other processes related to aging (Ferrari and Torres 2003), and the control which the antioxidants exercise over them, turn the antioxidant vitamins into key functional ingredients. The mechanism of action of vitamin E against lipid auto-oxidation has been widely described (Kamal-Eldin and Appelqvist 1996). The most interesting aspect of this action is its integration into the complex antioxidant system where vitamin E acts in synergy with other species such as selenium, vitamin C, and β-carotene. These components complement the activity of vitamin E through different mechanisms such as its regeneration or restoration after it has carried out its antioxidant function (Niki and others 1982; Wefers and Sies 1988). The interrelationship with sulfur-containing amino acids appears to be more related to the requirement of these in the synthesis of glutathione which regenerates vitamin E. In the case of transition metals such as Fe^{+2} and Cu^{+} its relationship with them is in their capacity to catalyze the decomposition of peroxides producing more free radicals and reducing the antioxidant effect of vitamin E.

47.3.2 Functional Properties of Vitamin E

Currently, studies of vitamin E point to the involvement in other activities not related to its capacity as an antioxidant, and concentrate more on its functionality. Among them, they describe cellular signaling and the improvement in the immune response. The first activity is related to the regulation of protein kinase C and other proteins or enzymes implicated in atherogenesis, reducing cell proliferation (Mahoney and Azzi 1988; Boscoboinik and others 1991; Chan 1998;). In particular α-tocopherol promotes the segregation of prostacyclin, a powerful vasodilator and platelet aggregation inhibitor. The role of vitamin E in the prevention of cardiovascular diseases is due to the existence of pathways, sensitive to this vitamin, which modifies cellular adhesion and proliferation, an activity that is supplementary to the control of oxidative stress. In regards to its functionality on the immune system, studies in humans have found an association between vitamin E and the immune response. Although this effect seems to be related to a decrease, induced by vitamin E, in the production of free radicals and related species, the role of this vitamin is not totally defined (Calder and Kew 2002). A recent work (Brigelius-Flohé 2003) summarizes how the different forms of vitamin E interact in the synthesis and activity of the enzymes responsible for the metabolism of drugs and xenobiotics.

Since the main activity provided by vitamin E is as an antioxidant, studies on its metabolism have been focused on the determination of compounds produced after this activity. In the last decade new metabolites, such as the carboxyethyl-hydrochromans (CEHC) that

keep the structure of chroman intact and do not take part in an antioxidant process, have been discovered (Shuelke and others 2000). The function of these metabolites as well as the catabolic pathways they generate, are areas still being investigated regarding each specific form of vitamin E. In particular, γ-tocopherol exhibits anti-inflammatory properties and its metabolite, γ-CEHC, shows natriuretic activity (Jiang and others 2001). With the high consumption of γ-tocopherol in diets such as in North America, the differences in bioavailability as compared to other E vitamers and the previously mentioned activities have increased interest in this tocopherol.

47.4 VITAMIN D

Vitamin D encompasses a group of compounds, very similar in structure, which possess antirickets activity of which ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) are notable. The former is derived from a vegetable steroid, ergosterol, while the latter is produced in the body from its precursor 7-dehydrocholesterol. This fact establishes that vitamin D is seen as a prohormone, which is metabolized in the liver and kidneys to its active form ($1\alpha,25(OH)_2D_3$), it functions as a steroid hormone. The discovery of this vitamin is associated to the prevention of the rickets and initially its activity was confused with that of vitamin A. It was not until 1922 when McCollum and others showed that the antirickets factor was a new substance, different to vitamin A and called it vitamin D (McCollum and others 1922). The need for ultraviolet (UV) light to obtain this vitamin, although sensed, was discovered years later. In 1970, the structure of the previously mentioned biologically active metabolite of vitamin D was determined.

Figure 47.4 shows the structure of the two main forms of vitamin D as well as their precursors and the metabolites $25(OH)D_3$ (which represents the vitamin D status in the individual) and $1\alpha,25(OH)_2D_3$, the active form. Starting from the cyclopentanoperhydrophenanthrene ring system present in the precursors and using UV radiation, a fission is produced in ring B which gives rise to a system of three double bonds, characteristic in all forms of vitamin D. Each particular form differs in the structure of the chain appended to the group of rings.

47.4.1 Nutritional Properties of Vitamin D

Vitamin D can be obtained from fortified foods, although its absorption efficiency is not very high, practically 50%, so that our body relies on the synthesis route to obtain the necessary amount of this vitamin. The Food and Nutrition Board sets a RDA of 5 μg/day for adults although this value is hard to specify due to the endogenous synthesis of this vitamin and its requirement depends on multiple factors (among them, the level of exposure to light, calcium, and phosphorous levels, development state of the individual). Vitamin D is found in appreciable quantities in fish such as salmon, sardines, and fish and liver oils. Other foods such as vegetables are poor sources of this vitamin; eggs, milk, and margarine contain very small amounts. For this reason, the fortification of foods such as pasta and cereals with vitamin D had become a normal practice for years and had almost eliminated rickets in the United States (Welch and others 2000). However, recent studies show a reemergence of deficiency in this vitamin and additional fortifying has been recommended (Moore and others 2004). The population groups in which this deficiency is most common are children, the elderly, and the obese. The lack of

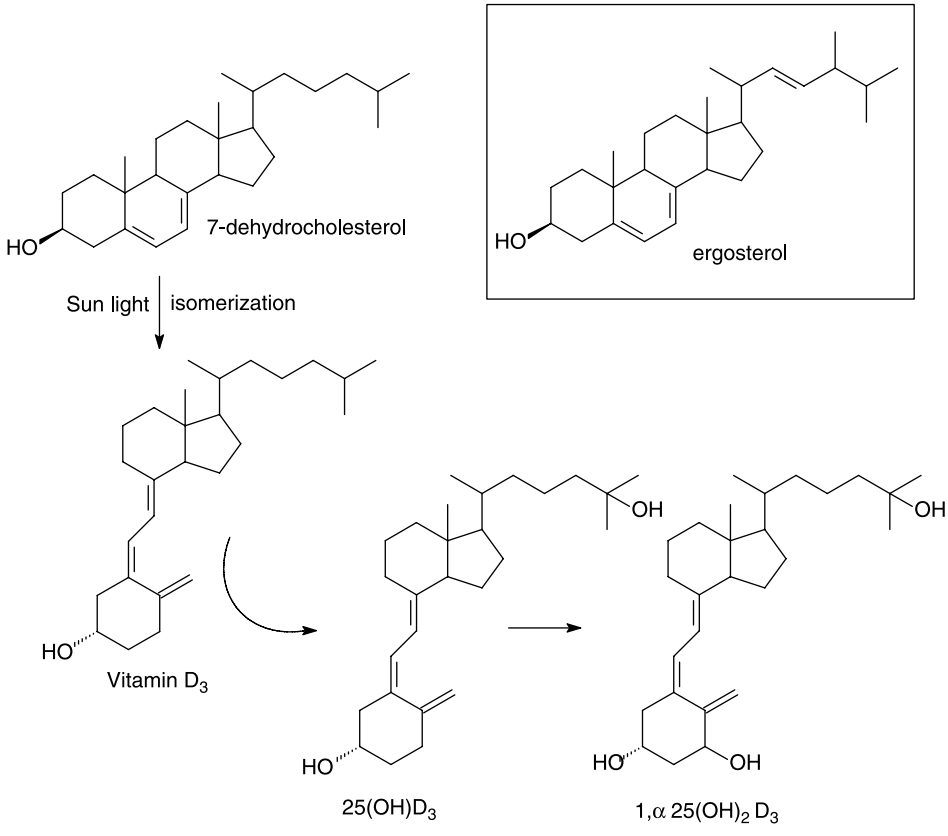


Figure 47.4 *Structure of previtamin D compounds 7-dehydrocholesterol and ergosterol, vitamin D_3, and the metabolites 25(OH)D_3 and 1α,25(OH)$_2D_3$.*

supplementation during breastfeeding (maternal milk does not contain vitamin D), lifestyle, and the loss of bioavailability are the main reasons why each population group suffers from vitamin D deficiency, which is also, very frequently, poorly diagnosed (Glerup and others 2000).

Vitamin D is principally involved in the maintenance of calcium and phosphorous homeostasis, and the health of the bones. Deficiency reduces the efficiency of calcium absorption (absorption is decreased by 10–15%), resulting in a drop in its concentration in the blood, which triggers off the mobilization of this mineral from the bones (Underwood and DeLuca 1984). Some osteoblast functions are regulated by the active metabolite of vitamin D, such as their proliferation, and the production of collagen and osteocalcin (Kurihara and others 1984; Pan and Price 1984).

47.4.2 Functional Properties of Vitamin D

Two decades ago the existence of nuclear receptors specific for vitamin D was verified. Since then an endocrine system associated with this vitamin has been defined, which has general effects over cell regulation and differentiation, effects not related to the maintenance of calcium homeostasis. Many degenerative processes are related to vitamin D

status, not only those that affect the bones, but also others involving the skin, liver, kidneys, intestine, pancreas, and lungs. Initially only the first were associated with its production, until the discovery of a large variety of tissues able to produce $1\alpha,25(OH)_2D_3$ (Cross and others 2001; Tangpricha and others 2001). Recently Lin and others (2002) demonstrated the existence of 150 genes regulated by this vitamin, and involved in very different functions such as the cell cycle, modulation of the redox system, and cellular signaling. Both factors highlight the importance of vitamin D in the handling of cell differentiation and proliferation which has ended up in implicating this vitamin with the prevention of cancer. This activity is also associated with the role of this vitamin in the regulation of the immune system, affecting the activity of T and B lymphocytes (Rigby and others 1985; Lemire 1992).

The biological functions of this vitamin-hormone carried out in our body closely related with the maintenance of health. There is also a reappearance of vitamin D deficiency states in particular population groups. Therefore, the functional foods have a decisive role in alleviating the possible deficiencies, producing a suboptimal health state that will become apparent immediately or in the longer term. For example, children with vitamin D deficiency are more prone to developing illnesses later in life such as type 1 diabetes, multiple sclerosis, and cancer (Holick 2004). A moderate exposure to the sun and the consumption of foods enriched with vitamin D, such as milk, juices, cereals, and fish oils are recommendations to be instilled into the population in general.

47.5 VITAMIN K

In 1935 Henrik Dam proposed, in two research articles, the existence of a new, lipid soluble, essential component, involved in the coagulation of blood, which he called vitamin K (Dam 1935a,b). Its existence enabled the discovery of the proteins involved in blood coagulation (prothrombin and fibrinogen). It was not until 1950 that the factors involved in the regulation and production of thrombin were defined. Subsequently, other factors (VII, IX, and X) also dependent on vitamin K for their synthesis were discovered.

The term vitamin K is designated to a group of compounds that have a common 2-methyl-1,4-naphthoquinone nucleus and are differentiated in the structure of the side chain in position 3. Figure 47.5 shows the structure of the three large groups of substances which exhibit anti-haemorrhagic activity. Vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) is preferably called phylloquinone. A group of compounds which constitutes another member of K vitamins (such as menaquinone-7) and presents unsaturations in the side chain, is denoted multiprenyl-menaquinones (vitamin K_2). And finally, 2-methyl-1,4-naphthoquinone, vitamin K_3, is correctly named menadione.

47.5.1 Nutritional Properties of Vitamin K

The unequivocal role of vitamin K in health is the maintenance of normal blood coagulation. It functions similarly to that of a co-enzyme involved in the synthesis of a group of proteins, which include the clotting factors II, VII, IX, and X, and the anticoagulant proteins C and S. Its activity is also fundamental for the synthesis of proteins involved in bone metabolism, such as osteocalcin and MGP (matrix gamma-carboxy-glutamic acid protein) which regulate the growth and deposition of minerals in the bones. The requirement for

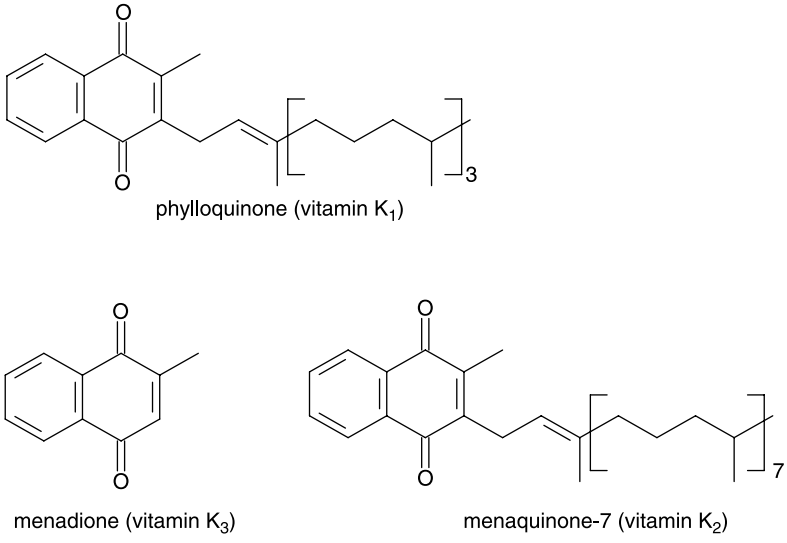


Figure 47.5 *Structure of the three groups of substances which exhibit antihaemorrhagic activity: phylloquinone, multiprenyl-menaquinones, and menadione.*

vitamin K in adults is extremely low. The Food and Nutrition Board establishes an adequate intake (AI) of 120/90 μg/day (males/females) without determining a maximum ingestion level. The risk of deficiency of this vitamin is very rare and a normal diet ensures reaching the recommended levels of ingestion. The development of high performance liquid chromatography has enabled the configuration of suitable tables which give the vitamin K content in common foods (Booth and others 1994, 1995). In general, the major sources of this vitamin are green vegetables along with edible oils. The only group at risk is the newborns; although rarely, they develop vitamin K deficiency bleeding. However, some authors recognize that this risk constitutes a public health problem (Shearer 1995). For this reason it is common practice to give oral supplements of vitamin K to newborns as well as fortifying baby foods with this vitamin so as to provide an intake higher than that of breast milk.

47.5.2 Functional Properties of Vitamin K

The involvement of vitamin K in the metabolism and health of the bones is receiving increasing attention and it is even being suggested that the recommended intake levels are not adequate for this vitamin to carry out its functional role. Some studies associate an increase in the risk of bone fractures and a decrease in mineral density with high levels of undercarboxylated osteocalcin, thus putting forward the hypothesis of a suboptimal vitamin K physiological state in relation to bone health (Bügel 2003). Shearer (1997) suggested that the carboxylation of nonhepatic Gla proteins is more susceptible to dietary influences than those proteins involved in blood coagulation. All this confers an important role of vitamin K in the pathogenesis of osteoporosis.

For this reason, techniques have been developed for determining vitamin K status based on functional markers, the undercarboxylated species of the vitamin K-dependent proteins being the most important markers. Vermeer and others proposed that

concentration of undercarboxylated species of osteocalcin is a reflection of an optimal state or deficiency of vitamin K (Vermeer and others 1995). The advantage of this procedure is to detect whether a deficiency of this vitamin exists in a specific tissue, such as bone, so that the needs of an individual or population group could be defined with greater accuracy, in order to decrease the risk of degenerative processes affecting that tissue in particular.

As was commented earlier, adequate databases on the vitamin K content of foods have not been developed until recently. Perhaps the fact that vitamin K requirements are very low, has brought about a certain lack of interest in the compilation of data. The functional role of this vitamin, the possibility that population groups need to increase their intake, and the discovery of new functional foods (which have a higher concentration of this micronutrient) are aspects, which require a currently expanding study.

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48

Phytochemicals in Mediterranean Diet: The Interaction Between Tomato and Olive Oil Bioactive Compounds

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48.1 Introduction 55
48.2 Tomato and Olive Oil Phytochemicals 58
48.3 Physiological Effects 60
48.4 Conclusions 62
References 63

48.1 INTRODUCTION

The word "phytochemicals" is commonly used to indicate thousands of chemically different natural metabolites used in medicine and food preparations (Table 48.1). Natural medicines have been used from the infancy of human history for their

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TABLE 48.1 Main Classes of Phytochemicals (Craig 1997).

Phytochemicals	Foods
Allyl sulfides	Garlic and onion
Phytates	Grains and legumes
Glucarates	Citrus, grain, and solanaceous vegetables
Lignans	Flax and soybeans
Isoflavones	Soybeans
Saponins	Legumes
Indoles, isothiocyanates, and dithiolthione	Cruciferous vegetables
Ellagic acid	Grapes, strawberries, raspberries, and nuts
Phthalides and polyacetylenes	Umbelliferous vegetables
Flavonoids, carotenoids, and terpenoids	Various plants and vegetables
Other phenolic compounds	Various plants and vegetables

pharmacological activity against many diseases or as simple folk remedy. Traditionally these medicines were obtained from plants, herbs, roots, and seeds, which could be used in their primary form or combined into mixtures. Nowadays, these preparations can be also formulated in pills, tablets, or liquids and are commercially available as natural medicines especially in Asian countries (Table 48.2).

Traditional Chinese medicine uses antioxidant-rich medicinal plants for the prevention of many diseases. These medicinal plants have a concentration of phenolic compounds

TABLE 48.2 Plants Found in Herbalists' Shop in Jordan (Afifi and others 2000).

Family Name	Scientific Name	Parts Used	Recommended Uses
Boraginaceae	Echium judaeum	Roots	Nervosity, hyperactivity, general weakness, dermatological disorders
Cruciferae	Brassica campestris	Seeds, leaves, roots	Hypercholesteremia, inflammation
Cucurbitaceae	Cucurbita maxima	Seeds	Ulcer, diabetes
Fagaceae	Quercus coccifera	Fruits	Kidney sands and stones, post delivery syndrome
Labiateae	Menta piperita	Leaves, stem	Common cold, cough, influenza, constipation, nervosity
	Ocium basilicum	Leaves	Common cold, cough, influenza, kidney sands, and stones, gynaecological disorders, alopacia
Leguminosae	Glycyrrhiza glabra	Roots	Common cold, cough, influenza, constipation, kidney sands, and stones
Liliaceae	Aloe vera	Leaves, juices	Abdominal pain, diabetes, weaning
Punicaceae	Punica granatum	Fruits, roots, and stem coat	Common cold, cough, influenza, ulcer
Rosaceae	Rosa damescana	Flowers	Common cold, cough, influenza, abdominal pain, gall-bladder stones, general weakness
Rutaceae	Citrus aurantium	Leaves, flowers fruits, peel, fruits	Weakness in myocardium, gynaecological disorders
Umbrelliferae	Petroselinum sativum	Leaves, steam	Urinary tract infections, arthritis

TABLE 48.3 Protective Effects Reported for Soy Isoflavones.

Cancer	Epidemiological studies show a protective effect of soy consumption against hormone-dependent cancer of the breast and prostate (Barnes and others 1998) and against bowel, stomach, olon, rectum, and lung cancer (Messina and Messina 1991; Messina and others 1994; Adlercreutz and others 1995; Herman and others 1995)
Heart disease	Soy protein has a hipocholesterolemic effect (Fukui and others 2002) and may reduce the risk of atherosclerosis (Anderson and others 1995; Potter 1995) and of coronary heart diseases (Anderson and others 1999; Hermansen and others 2001; Nicolosi and others 2001; Hasler 2002). Isoflavones are thought to be an important hypocholesterolemic component in soy protein (Anthony and others 1996,1997; Kirk and others 1998; Ni and others 1999)
Osteoporosis	Isoflavones protect against osteoporosis (Potter and others 1998; Wiseman 2000; Scheiber and others 2001)
Antioxidative effect	Isoflavones acts as antioxidants and scavengers of reactive oxigen species (ROS) and reactive nitrogen species (RNS) (Arora and others 1998; Yen and Lai 2003)
Other	Soy isoflavones also exhibit biological activities such as inhibition of cell proliferation (Coward and others 1993; Fotsis and others 1995) and enzyme-inhibitory effects (Keung and others 1993)

(phenolic acids, flavonoids, tannins, coumarins, lignans, quinines, stilbenes, and curcuminoids) significantly higher than vegetables and fruits (Cai and others 2004).

The dietary intake phytochemicals in Western countries is related more to the consumption of fruits and vegetables than to the use of specific medicinal plants or extracts. In the last decade soybeans and related products, traditionally consumed especially in Asia, have been conquering Western markets thanks to their biological effects on humans. The positive effects on health exerted by these foods (Table 48.3), previously attributed only to their high isoflavones content (genistein, daidzein, glycitein), are recently thought to be due to the combination of many compounds such as proteins, amino acids, peptides, saponins, phytic acids, trypsin inhibitors, fiber, and isoflavones (Barnes and others 1998; King and Bignell 2000; Fukui and others 2002).

Beside the bioactive vegetable compounds naturally present in foods and medicinal plants, the phenomenon of phytochemical-rich ingredients used as components of commercial food preparation is becoming of nutritional relevance. Many phytochemicals have been used by food industries to increase the shelf-life of their products. For example, meat industries largely add phytochemical mixtures to prevent the oxidation of its products.

Lipid oxidation is the most important factor implicated in meat spoilage during storage, and thus in the reduction of its shelf-life due to off-flavor and off-color development (Pearson and others 1983). The losses of nutritional value and functionality (Matsushita 1975) could be reduced using synthetic antioxidants (BHA, BHT, and citric acid) but many investigations performed in model systems showed that natural antioxidants are as effective as synthetic antioxidants in retarding oxidation (MacNeil and others 1973; Madsen and Bertlesen 1995). Nissen and co-workers (2000) showed that rosemary extract had the same efficacy of BHT and octyl gallate in protecting the dehydrated chicken meat against oxidative deterioration, followed by tea and coffee extract, while grape skin extract was the less efficient among the tested extracts. Moreover, consumers are more willing to accept natural extracts than synthetic antioxidants.

Addition and integration of phytochemicals, especially antioxidants, for technological and nutritional purposes is a fascinating field of food technology. From this point of view

many traditional recipes of the Mediterranean diet give unexpected opportunities when the behavior of antioxidant compounds during the technological processes is considered. In Mediterranean countries the large consumption of tomatoes and virgin olive oil, which are present in many traditional recipes, ensures a high intake of antioxidant phytochemicals, such as carotenoids and polyphenols, respectively. This is due to the high natural occurrence of these compounds, but also, as we will show in the following pages, to the type of processes usually adopted for the preparation of these food.

In this chapter we discuss phytochemicals present in these staple foods of Mediterranean diet, tomato and olive oil, focusing our attention on their natural content in raw materials and the positive influence of technological processes from the nutritional point of view.

48.2 TOMATO AND OLIVE OIL PHYTOCHEMICALS

The widespread use of tomatoes and tomato-based products in Mediterranean diet make this fruit an important source of minerals, vitamins, and healthy phytochemicals such as carotenoids and flavonoids. The concentration of secondary metabolites in tomatoes depends on many factors. Carotenoid biosynthesis is influenced by the variety, cultivation area, stage of ripening at harvest, agronomical practices (i.e., irrigation, fertilization, etc.), and the storage conditions. Leonardi and co-workers (2000) reported that the concentration of carotenoids in commercial tomatoes ranges from 0.6 to 13 mg per 100 g of green salad and full ripe cherry tomatoes, respectively. On the other hand, flavonoid biosynthesis is poorly correlated with ripening stage, while it seems more dependent on climatic and solar radiation conditions; in fact, tomatoes leading to the same variety results richer in flavonoids when grown in Mediterranean area than fruits cultivated in North Europe (Stewart and others 2000).

Since tomatoes are an excellent source of carotenoids, but a modest source of flavonoids, there is considerable interest in the production of fruits containing increased levels of flavonoids with a consequent wider range of potential health beneficial properties. Several breeding companies and research institutes obtained tomato mutants containing higher levels of carotenoids and flavonoids than commercial varieties.

Tomato micronutrient content is also influenced by industrial process, which represents another important factor determining antioxidant intake considering that more than 75% of tomatoes consumption derives from processed products. All tomato-based products undergo a more or less severe thermal treatment, which can affect the stability of tomato bioactive compounds. It is generally accepted that carotenoids are poorly affected by processing; on the other hand, data for flavonoids are not conclusive, also because flavonoids are mainly located in the skins, which are usually discarded in processed tomatoes. In fact, while literature data (Crozier and others 1997; Stewart and others 2000) report that industrial canning causes a severe reduction of flavonols content, a recent work performed by our group showed that, when tomatoes are canned without the previous peeling, no thermal degradation of flavonoids was observed (Pernice and others in press). As far as olive oil phytochemicals the absolute concentration of phenolic compounds is the result of complex interactions between several factors, including cultivar, ripening degree, climate, and extraction process (Caponio and others 1999; Fogliano and others 1999). The amount of phenolic compounds is also affected by oxidative and hydrolytic modification during storage (Della Medaglia and others 1996).

TABLE 48.4 Effect of Heating Time on Lycopene Concentration in Peeled Tomato Puree and in Peeled Tomato Puree Added of 5% Virgin Olive Oil.

	Lycopene Concentration (mg/100 g of Dry Matter)	
Heating Time	Peeled Tomato Puree	Peeled Tomato Puree + 5% Virgin Olive Oil
0	61.12 ± 3.7	70.37 ± 5.5
0.5	59.26 ± 3.7	62.96 ± 5.6
1	55.56 ± 1.8	64.81 ± 7.1
2	53.70 ± 5.5	62.95 ± 3.7
4	46.30 ± 1.8	59.26 ± 3.6
9	37.04 ± 3.7	59.26 ± 3.8

The phenolic fraction consists of the so-called secoiridoid derivatives, formed by *p*-hydroxyphenylethanol (*p*-HPEA) or by dihydroxyphenylethanol (DHPEA) linked to elenolic acid. Phenolic compounds, particularly *o*-dihydroxy derivatives, have strong antioxidative activity and are essential to preserve the fatty acid moiety of VOO from oxidative damage during processing and storage (Baldioli and others 1996; Litridou and others 1997). Furthermore, *o*-dihydroxyphenolic compounds contribute to the stability of the oil, due to their ability to donate a hydrogen atom and form an intramolecular hydrogen bond between their hydroxyl group and phenoxyl radicals (Visioli and Galli 1998).

In the last few years, we have investigated the effect of industrial process and home cooking on the interactions between carotenoid and flavonoid of tomatoes and polyphenol of virgin olive oil in tomato sauces. Home-made tomato sauces are prepared by mixing 3–5% of extra-virgin olive oil to fresh or canned tomatoes and cooking them for a period ranging from few minutes to many hours, as it happens in traditional recipes of southern Italy. During thermal treatment phytochemicals degradation could be expected. However, the presence of a two phase-system (oil-in-water), causes a peculiar phase partition of virgin olive oil polyphenols, as well as tomato carotenoids and flavonoids.

A model system study (Graziani and others 2003) showed that, heating in laboratory-scale conditions, a mixture of tomato and 5% olive oil, the latter had a protective effect on tomato antioxidants (Table 48.4). Lipid matrix favored the extraction and the detection of tomato carotenoids (Fig. 48.1) and at the same time olive oil phenolic

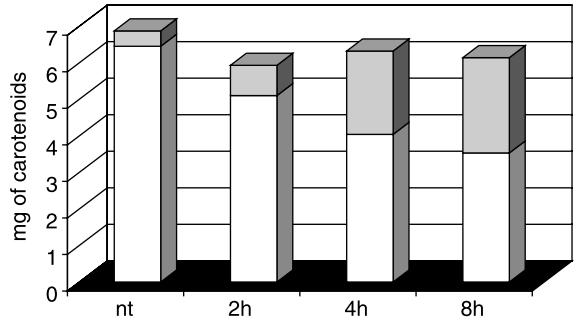

Figure 48.1 *Effect of heating time on carotenoid repartition in an oil-tomato mixture. The gray bars correspond to oil carotenoids while the white ones correspond to tomato carotenoids; "nt" is for not thermal treated samples.*

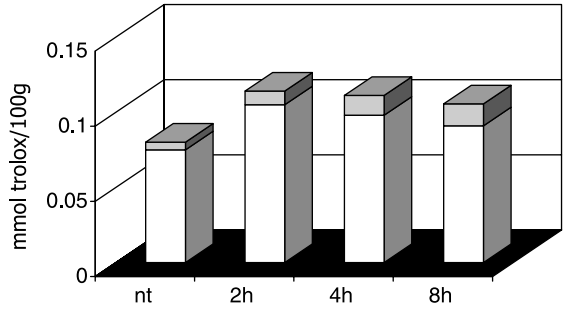

Figure 48.2 *Antioxidant activity of water and oil phases measured during heating in a tomato olive oil system. Note: The grey bars correspond to oil antioxidant activity while the white ones correspond to tomato antioxidant activity; "nt" is for not thermal treated samples.*

compounds protected carotenoids from oxidation thus resulting in an enhanced antioxidant activity of the heated mixture (Fig. 48.2). Accordingly, Anese and co-workers (2002) showed that using industrial-like treatment on tomato–olive oil system there were no significant change in lycopene concentration.

The positive interaction between the various classes of antioxidants in this system have implications in the stability and storage of tomato sauces, and these findings are very important from the stand point of the physiological effects that these compounds may have on humans.

48.3 PHYSIOLOGICAL EFFECTS

The *in vivo* physiological effects of carotenoids are related to their bioavailability. Many studies demonstrated that the uptake of carotenoids is greater from heat processed than from unprocessed tomato, and that the absorption efficiency is also affected by the contemporary presence in the diet of other food components such as dietary fats and proteins (Dimitrov and others 1988; Rock and Swendseid 1992; Stahl and Sies 1992; Gärtner and others 1997). Giovannucci and co-workers (1995) found that the intake of tomato sauce was significantly correlated with lycopene concentrations in plasma, while the administration of tomato juice did not cause any increase of lycopene plasma concentrations. A slight increase was also correlated to the intake of fresh tomatoes. These findings were attributed to the common practice to consume tomato sauce and fresh tomatoes together with oil. The fat matrix has two positive effects on carotenoid bioavailability: in the stomach it favors carotenoid extraction from food matrix. In the upper gut the presence of oil stimulates the excretion of biliary acids, the consequent formation of chylomicrons and therefore the absorption of all lipophilic phytochemicals.

On the basis of this consideration an antioxidant-rich functional food (FF) constituted by a tomato puree mixed with 10% extra-virgin olive oil was produced in an industrial plant and a pilot bioavailability study of carotenoids was performed. For this purpose five healthy volunteers (2M and 3F) that were asked to consume 100 g/day of the FF (23 mg carotenoids/day) for a week were enrolled. The control group was constituted of three volunteers (2M and 1F) that did not consume the FF. Blood samples were drawn before (T_0), after a week of FF administration (T_7) and after a week of wash-out (T_{14}). Serum samples collected were analyzed for carotenoid composition. In particular

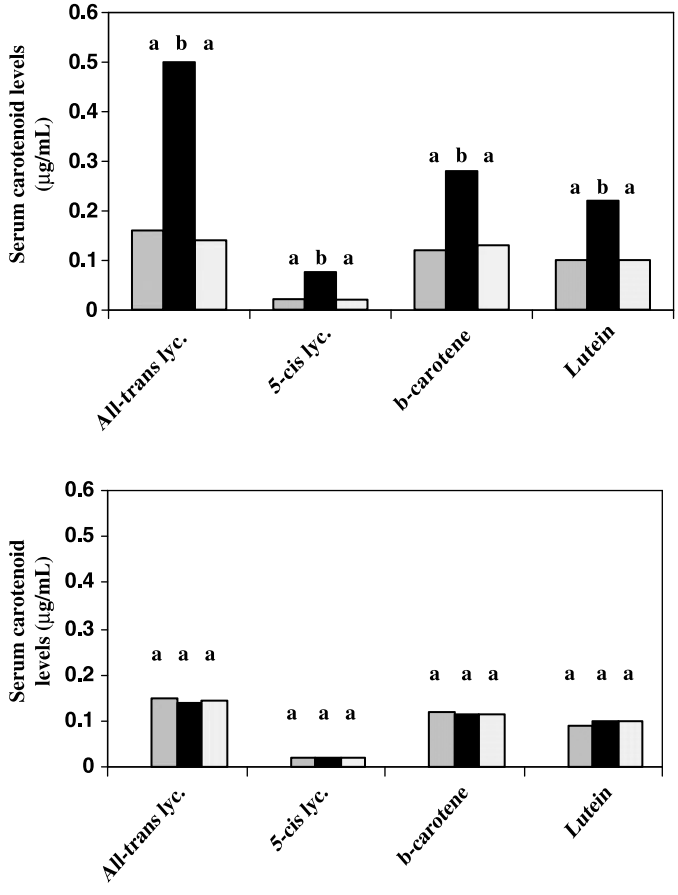

Figure 48.3 *Top Panel: average serum carotenoid concentration in subjects that consumed the FF. Bottom Panel: average serum carotenoid concentration in subjects of control group. Shaded bars T_0; black bar T_7; pointed bar T_{14}.*

all-trans- and *5-cis*-lycopene, lutein and β-carotene were quantified by HPLC as described by Holloway and others (2000).

The results (showed in Figure 48.3) demonstrate that in all the subjects that consumed the FF, an increase of the bioactive compounds directly related to FF antioxidant contents was found. In particular after the week of administration, β-carotene and lutein levels doubled the basal levels (0.28 ± 0.17 μg/mL vs. 0.12 ± 0.09 μg/mL and 0.22 ± 0.13 μg/mL vs. 0.10 ± 0.08 μg/mL respectively) while *all-trans* lycopene levels were four-fold higher (0.50 ± 0.15 μg/mL vs. 0.16 ± 0.03 μg/mL). After the wash-out period the carotenoid serum concentration come back to the basal levels ($p < 0.05$).

Heating tomatoes in the presence of fat increases the bioavailability of carotenoids, but it is important to underline that the composition of fat can also affect this parameter. On this regard, Lee and others (2000) carried out a study to value the effect of cooking tomatoes with two different oils, extra-virgin olive oil and sunflower oil, on plasma lycopene concentration and plasma antioxidant activity. Six subjects (5F and 1M) were asked to consume for a week, 200 g of tomato soup (33 mg lycopene) and 230 g of

TABLE 48.5 Effect of Supplementation of Tomato Products with Olive Oil and Sunflower Oil (Lee and others 2000).

	Tomato Products with Olive Oil		Tomato Products with Sunflower Oil	
	Baseline	Week 1	Week 4	Week 5
Dietary lycopene	<5	46 ± 10	<3	46 ± 12
Plasma lycopene (μmol/L)	0.66 ± 0.26	1.20 ± 0.20	0.67 ± 0.27	1.14 ± 0.35
Plasma antioxidant activity (μmol/L)	930 ± 150	1118 ± 184	1049 ± 186	1009 ± 181

canned tomatoes (13 mg lycopene) with 20 mL of extra-virgin olive oil, followed by three weeks of washout, then they were supplemented for another week with the same amount of tomato products plus 20 mL of sunflower oil. The results (Table 48.5) showed that the supplementation of tomato products (about 46 mg/day of lycopene) with olive oil and sunflower oil produced respectively 80% and 70% increase of the plasma lycopene levels. The increase was not significantly different between the two oils. On the contrary, plasma antioxidant activity (measured with FRAP assay) increased only with consumption of tomato products with olive oil, while with sunflower oil the plasma antioxidant activity is slightly below the control level. Again, this result is likely due to the amazing integration between the antioxidant component of olive oil and that of tomato.

48.4 CONCLUSIONS

The link between diet and chronic diseases is very well documented. Medical research is focused on prevention of major chronic diseases to reduce the current high cost of medical care. As a consequence, nowadays major attention is referred to food components note for their capability to exert positive effects on human health, such as phytochemicals, whose activities have been demonstrated by many scientific studies. Among phytochemicals, antioxidants have been hypothesized to play a major role as an enhanced production of free radicals and/or significant decrease of antioxidant defence is correlated to many diseases (Weisburger 1999). Moreover, antioxidant-rich foods have been shown more effective than pills, tables and other synthetic nutraceuticals.

In recent years, the consumer demand of enjoyable foods that are able to satisfy senses, but also maintain an healthy status, is continuously increasing. This tendency is coupled with the large diffusion of Mediterranean diet in the world, as this alimentary regime is considered a model for a healthy diet. Mediterranean diet is characterized by a greater intake of cereal, fruit, and vegetables respect to animal-derived foods, thus assuring a large intake of bioactive compounds. Among the staple foods of Mediterranean diet tomatoes and virgin olive oil represent an optimal antioxidant mixture by nutritional and technological point of view.

The phytochemicals presence in raw foods and their capability to undergo to the industrial processing should be taken in serious account by the food industry. In fact, the selection of antioxidant-rich raw foods, and better control of processing conditions could ensure the highest bioactive compound content in the final products, thus increasing the functionality of the offered products also thanks to a particular interaction and synergism among food compounds that improve the bioavailability (Beecher 1998). As consequence the bioavailability of phytochemicals used as food ingredients, and the choice of food matrix, must be carefully considered.

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49

Functional Microbes: Technology for Health Foods

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49.1 Rationale for the Development of GI Tract Targeting Functional Foods 67
49.2 Requirements for an Effective Probiotic 68
49.3 Health Claims Attributed to Probiotics 70
49.4 Mixtures of Pro- and Prebiotics, Synbiotics 71
49.5 Probiotic Technology: Maintaining and Enhancing the Functionality and Viability of Microbes with Health Benefits 71
49.6 Probiotic Food Market: "Healthy Foods are Here to Stay" 74
References 78

49.1 RATIONALE FOR THE DEVELOPMENT OF GI TRACT TARGETING FUNCTIONAL FOODS

There is a growing demand for developing foods with specific functionalities increasing the health and well-being of the consumers. This need originates from the notion that consumers at critical ages, especially children and elderly, are prone to food-related diseases

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and gastrointestinal (GI) disorders. The symptoms of many GI disorders and diseases can be alleviated and prevented by consuming foods with specific functionality for health (see references in Table 49.1).

Emerging scientific developments enable the rational development of foods with increased functionality. This notably holds for foods that target the GI tract, the primary site of food conversion and uptake, where food-borne or systemic disorders are abundant, and the innate immune system is stimulated. This body's metabolically most active organ is colonized by a myriad of microbes that contribute to food conversion, communicate with the host, and induce specific responses that contribute to a wide variety of important physiological functions. Hence, the gut represents the site where the host's well-being is affected by foods either directly or by modulating the interplay between microbes and host (Salminen and others 1998).

The scientific development in the gut-health area is also facing challenges. This includes the genomics-related and high throughput molecular tools aimed to provide insight into the molecular mechanisms of food functionality, gut health, and microbial function. Insight in the basic mechanisms of gut health is required to further develop biomarkers for probiotics (live microbial food supplements with a beneficial effect on human health: Anon. 2002), as well as prebiotics and other dietary components that stimulate specific microbial groups in the human gut (Vaughan and others 2000).

Furthermore, the development of functional foods includes a major investment on technology development. Probiotic functional foods face high technological demands because probiotics have to retain their viability during all the production steps and even in the GI tract of the consumer. Therefore the new manufacturing process and formulation technologies are often required for probiotic bacteria primarily selected for their functional health properties (Saxelin and others 1999; Saarela and others 2000).

49.2 REQUIREMENTS FOR AN EFFECTIVE PROBIOTIC

Microorganisms used for health purposes are generally classified as either probiotics or functional foods in the EU or as biotherapeutic agents or dietary supplements in the United States. Unfortunately, the use of a specific term in the product label does not provide any basis by which the consumer can judge the functional validity of the product. Although lactic acid bacteria (LAB) have a long history of use in Europe and Asia, the scientific evidence for their efficacy and safety has not been rigorously addressed until fairly recently. Scientific credibility of these products will only evolve after well-controlled clinical trials in humans are performed, up-to-date methodologies are used, and conclusive evidence of benefits is obtained.

In general, probiotic microbes have been given to humans in the form of encapsulated lyophilized powder or in a food vehicle (e.g., fermented or nonfermented dairy products or fruit/berry juices). The types of microbes tested for their probiotic potential include *Lactobacillus* sp. (*L. acidophilus*, *L. reuteri*, *L. casei*, *L. johnsonii*, *L. plantarum*, *L. rhamnosus*), *Bifidobacterium* sp. (*B. bifidum*, *B. infantis*, *B. animalis/lactis*, *B. longum*, *B. breve*), some other bacteria (*Enterococcus faecalis*, *Escherichia coli*, and *Bacillus cereus*) and yeasts (*Saccharomyces boulardii*, *S. cerevisiae*) (Alvarez-Olmos and Oberhelman 2001; Table 49.1).

For a microbe to become a successful biotherapeutic agent, several important requirements need to be fulfilled. The most critical are survival to the active site, association with

TABLE 49.1 Clinical Effects of Some Probiotic Strains.

Strain	Clinical Effects in Humans	References
Lactobacillus rhamnosus GG (ATCC 53103)	Lowering fecal enzyme activities, reduction of antibiotic-associated diarrhea in children, treatment and prevention of rotavirus and acute diarrhea in children, treatment of relapsing *Clostridium difficile* diarrhea, reduces the symptoms of antibiotic treatment of *H. pylori*, immune response modulation, alleviation of atopic dermatitis symptoms in children, prevention of the risk of allergy in infancy, reduces the severity of respiratory infection in children	Siitonen and others 1990; Goldin and others 1992; Kaila and others 1992; Hosoda, others 1994; Isolauri and others 1991, 1994, 2000; Majamaa and others 1995; Raza and others 1995; Sepp and others 1995; Bennett and others 1996; Malin and others 1996; Hilton and others 1997; Majamaa and Isolauri 1997; Shornikova and others 1997; Alander and others 1997, 1999; Pelto and others 1998; Rautanen and others 1998; Arvola and others 1999; Oberhelman and others 1999; Vanderhoof and others 1999; Armuzzi and others 2001a,b; Hatakka and others 2001; Kalliomäki and others 2001, 2003; Näse and others 2001; Szajewska and others 2001; Cremonini and others 2002; Rautava and others 2002
Lactobacillus johnsonii (*acidophilus*) LJ-1 (La1, LC-1)	Modulation of intestinal flora, immune enhancement, down-regulation of *H. pylori* infection and gastritis, decrease of *H. pylori* colonisation in children	Link-Amster and others 1994; Schiffrin and others 1995; Marteau and others 1997; Michetti and others 1999; Donnet-Hughes and others 1999; Felley and others 2001; Cruchet and others 2003
Bifidobacterium animalis subsp. *lactis* Bb-12	Prevention of traveller's diarrhea, treatment of viral diarrhea including rotavirus diarrhea, modulation of intestinal flora, improvement of constipation, modulation of immune response, alleviation of atopic dermatitis symptoms in children	Black and others 1989, 1991; Marteau and others 1990; Alm and others 1993; Link-Amster and others 1994; Saavedra and others 1994; Schiffrin and others 1995; Fukushima and others 1998; Haschke and others 1998; Isolauri and others 2000
Lactobacillus casei Immunitas (DN-114001)	Reduction of the duration, severity and incidence of diarrhea in children, reduction of the duration of winter infections in elderly, lowering fecal enzyme activities	Guerin-Danan and others 1998; Pedone and others 1999, 2000; Agarwal and Bhasin 2002; Turchet and others 2003
Lactobacillus casei Shirota	Modulation of intestinal flora, lowering fecal enzyme activities, positive effects on superficial bladder cancer and cervical cancer, immune response modulation, adjunctive therapy of chronic constipation	Aso and Akazan 1992; Okawa and others 1993; Tanaka and Ohwaki 1994; Aso and others 1995; Spanhaak and others 1998; Nagao and others 2000; Ohashi and others 2002; Koebnick and others 2003
Lactobacillus plantarum DSM 9843 (299v)	Modulation of intestinal flora, increase in fecal short-chain fatty acid content, lowering of fibrinogen and cholesterol levels, reducing the symptoms of IBS, reduction in cardiovascular disease risk factors in smokers	Johansson and others 1993, 1998; Bukowska and others 1998; Nobaek and others 2000; Niedzielin and others 2001; Naruszewicz and others 2002

mucosal surfaces, ability to survive in the presence of normal flora, survival in the presence of metabolic substances and enzymes, along with the production of a beneficial action or product (McFarland and Elmer 1997).

Survival of probiotic bacteria through the upper gastrointestinal tract to reach the target site (usually the caecum or colon) has been documented in several publications (e.g., Goldin and others 1992; Marteau and others 1992; Johansson and others 1993, 1998; Alander and others 1997, 1999). The survival rates vary greatly, depending on a strain and preparation (e.g., capsule vs. fermented milk vs. fruit juice) used (Saxelin and others 2003). For example, in the study of Marteau and others (1992) two probiotics, *L. acidophilus* and a *Bifidobacterium* species, survived passage through the small intestine and the percent recovery of the oral dose was 1.5% for *L. acidophilus* and 37.5% for the *Bifidobacterium*. It is extremely important that in recovery experiments using therapeutic microbes, the strain that is given orally can be identified separately from resident microbiota. For this, highly discriminatory genetic fingerprinting techniques or strain-specific direct detection methods are essential.

49.3 HEALTH CLAIMS ATTRIBUTED TO PROBIOTICS

The health claims attributed to probiotics are broad. These include alleviation of lactose intolerance symptoms, treatment of viral and antibiotic associated diarrhea, treatment of infant gastroenteritis, reduction of symptoms of antibiotic treatment of *Helicobacter pylori*, alleviation of atopic dermatitis symptoms in children and prevention the risk of allergy in infancy, alleviation of symptoms of IBD (inflammable bowel disease) and IBS (irritable bowel syndrome), enhancing the immune response, modulation of intestinal microbiota, lowering the levels of harmful fecal enzymes, and positive effects on superficial bladder cancer and cervical cancer. There are also several potential effects, which have not been adequately established, including, for example, lowering the activity of rheumatoid arthritis, prevention of urinary tract infection, and so on (Tables 49.1 and

TABLE 49.2 Probiotic Health-Effects. Note that not all Effects can be Attributed to a Single Probiotic Strain (Saarela and others 2000, 2002; Reid and others 2003).

Established Effects	Potential Effects
Alleviation of lactose intolerance symptoms	Improvement of constipation
Treatment of viral (rotavirus) diarrhea	Antimutagenic/anticarcinogenic activity
Treatment of infant gastronteritis	Lower the activity of rheumatoid arthritis
Treatment of antibiotic associated diarrhea	Treatment of candidal and bacterial vaginitis
Alleviation of atopic dermatitis symptoms in children	Prevention of urinary tract infection
Preventing the risk of allergy in infancy	Treatment of cystic fibrosis
Alleviation of IBD symptoms	Protective agents in primary prevention of atherosclerosis in smokers
Alleviation of IBS symptoms	Lowering of cholestrol and blood pressure
Immune modulation	Eradication of multidrug-resistant microbes
Adjuvant in *H. pylori* treatment	Prevention of transmission of AIDS and sexually transmitted diseases
Lowering biomarkers (harmful fecal enzymes)	Infection control
Positive effects on superficial bladder cancer and cervical cancer	

49.2). Probiotics have been found to be most effective when an alteration of the normal microbiota is involved. Several studies of diseases involving the intestinal tract have found good clinical evidence of efficacy from placebo controlled clinical trials (see references in Table 49.1).

49.4 MIXTURES OF PRO- AND PREBIOTICS, SYNBIOTICS

A synbiotic has been defined as a "mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GI tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare" (Gibson and Roberfroid 1995; Salminen and others 1998). The use of a combination of a probiotic and a prebiotic (substrates that selectively stimulate the proliferation and/or activity of advantageous groups of bacteria already present in the intestinal microbiota) has distinct advantages. A problem with probiotics is their survival, both in the product and after ingestion, as most probiotics are only effective if viable (Fuller and Gibson 1997; McFarland and Elmer 1997; Ouwehand and Salminen 1998). The problem may be overcome by using the live microbial addition in conjunction with a specific substrate for growth (i.e., synbiotics). As such, improved survival and growth of the probiotic ought to occur. New product developments may more fully exploit the synbiotic approach. Although synbiotic approach holds a lot of promise, only a few clinical trials using synbiotic preparations have been reported so far (Kanamori and others 2002; Kiessling and others 2002; Anderson and others 2004).

49.5 PROBIOTIC TECHNOLOGY: MAINTAINING AND ENHANCING THE FUNCTIONALITY AND VIABILITY OF MICROBES WITH HEALTH BENEFITS

Probiotic production technologies should provide in a cost effective manner high-quality, safe end-products with long enough shelf-life. High quality in this connection mainly means products containing high numbers of living probiotics, but no contaminating microbes. For the food industry the easy formulation of probiotics into foods is of utmost importance. For this reason probiotics are usually provided as DVI (direct vat inoculation), which are highly concentrated and can be used at the production site without including a propagating step for probiotic cells (Gomes and Malcata 1999; Saarela and others 2000).

For the successful production of high-quality probiotic products a solid knowledge on the characteristics of the production strain is necessary. Working with living microbes is demanding since viability losses occur easily if the microbial cultures encounter stressful situations and conditions. Microbes can become stressed for several reasons: they have unique optimal growth conditions (nutrients, pH, gas atmosphere, temperature) and maintaining these optimal conditions in batch cultures in fermenters is difficult since, due to the growth and diminishing nutrient concentrations, their environment changes constantly (Rallu and others 1996). Various downstream processing steps of probiotic production unavoidably cause stress to microbes. These include, for example, harvesting, freezing, and drying. The main results of stress in cells is damage of various molecules, especially

important being the damage in proteins. If the stress is sublethal, microbial cells try to cope with it by producing so-called stress proteins (Cotter and Hill 2003). Some stresses lead into a situation where cells enter a nonculturable state (some of these can revert back to culturable and some not). However, if the stress is severe, loss of viability is unavoidable (Barer and Harwood 1999; Edwards 2000).

When working with probiotics their special features have to be kept in mind. Probiotics have usually been isolated from the human GI tract and they are well adapted to that special environment (nutrients, oxygen pressure, pH, temperature, etc.). Microbes that survive and thrive in the GI tract can therefore be difficult to propagate in the laboratory. However, to function properly, probiotic products should contain high enough levels of the specific probiotic strain(s) during the storage and consumption. Thus, great demands are imposed on probiotic production; probiotic strains have to be amenable to industrial-scale production and they have to survive and retain their functionality during storage as frozen or freeze-dried cultures, during formulation into foods and food storage, and finally in the GI tract.

Studies on the quality of commercial probiotic products have revealed deficiencies in a number of them especially regarding labeling and viability of probiotic strain(s). This problem is even enhanced in probiotic supplements such as capsules and powders, which often have both quantitative and qualitative problems (Canganella and others 1997; Hamilton-Miller and others 1999; Fasoli and others 2003; Temmerman and

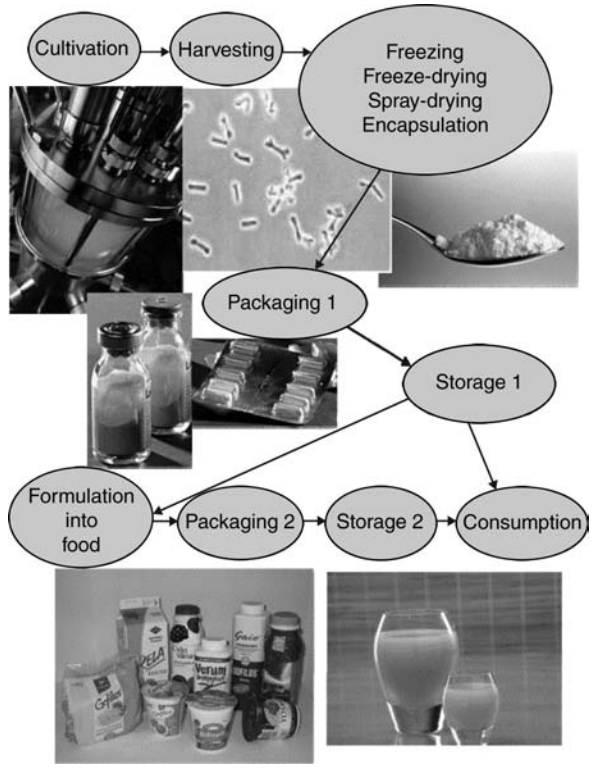

Figure 49.1 *Different steps of production and processing of probiotic products.*

others 2003a,b). These findings indicate that problems in producing probiotic products too often result in deficient quality of the end products. Typical problems are difficulties in obtaining high cell concentrations in fermenter scale and retaining viability during downstream processing. In addition, probiotics often have poor stability in the end product. Factors that effect probiotic viability during production and formulation include, for example, strain characteristics (even strains within species can show differences), nutrients or matrix to which the strain is formulated, pH, atmosphere, possible accompanying microbes, storage time, and temperature (Nighswonger and others 1996; Dave and Shah 1997; Kailasapathy and Rybka 1997; Micanel and others 1997; Gardini and others 1999; Saxelin and others 1999; Godward and others 2000; Saarela and others 2000; Vinderola and others 2000; Heller 2001; Oliveira and others 2001).

There are several steps of probiotic production where probiotic viability and stability can be enhanced. These steps include strain selection and improvement of strain properties, production conditions, formulation, and packaging and storage (Fig. 49.1). If possible, production strain should be chosen based both on functional properties and technological robustness. Since both functional and technological properties can vary remarkably within a genus and even within a species, a practical approach, which can save resources during the production step, is to screen for novel probiotic strains based on their technological properties. Among the technologically feasible strains, a strain with good functional properties should then be selected. However, in a situation when we have a functionally good but technologically poor strain in our hands we can attempt to improve its technological properties. This can be done in two ways; by activating the stress genes of the strain during processing with suitable sublethal treatments (van de Guchte and others 2002), or by genetically modifying the strain to make it more robust (e.g., by transfer, multiplication, and/or additional activation of genes responsible for stress response). Stress response in cells is inducible and nonpermanent. This means that the treatment has to be done immediately prior to harvesting and downstream processing.

Probiotic strains can vary in their requirements for nutrients, for suitable pH and gas atmosphere, fermenting, and harvesting time. During the propagation and downstream processing of probiotic cultures strain-specific demands have to be considered (Saxelin and others 1999). A suboptimal pH at the end of fermentation induces the stress response in the culture and can help it to survive better during downstream processing steps. The most suitable harvesting time is usually the early stationary phase. Cells that enter into stationary-phase develop a general stress-resistance and are thus more resistant to various types of stresses (including downstream processing and storage) than cells in the log-phase (Brashears and Gilliland 1995; Lorca and de Valdez 1999; van de Guchte and others 2002).

After harvesting cells are frozen, freeze-dried, spray-dried, or encapsulated. To enhance the survival of the culture during these steps protectants are needed. For the cryoprotection of LAB a large selection of compounds has been used. These include skim milk with or without supplements such as polyethylene glycol, dextran, bovine albumin, glycogen, sucrose, trehalose, glycerol; disaccharides such as sucrose, lactose, maltose, and trehalose; Me_2SO, glycerol, betaine, sodium ascorbate and glutamate, and maltodextrine (for a review see Hubalek 2003). Some of these compounds can also be used as thermoprotectants. For the LAB encapsulation carriers/supporting material used include alginate, carrageenan, cellulose acetate phthalate, chitosan, gelatine, gum arabic, and starch (Krasaekoopt and others 2003). Basic encapsulation techniques

are extrusion and emulsion, but cells can also be encapsulated during freeze- and spray-drying.

Unless probiotics are consumed as capsules or powders they are further formulated into food-stuffs. During this formulation step several things need to be considered such as the composition (nutrients, antimicrobials), structure (oxygen permeability, water activity), pH of the matrix, and interactions with starter microbes in fermented food matrices. Growth of probiotics in nonfermented foods is not desirable (due to possible off-flavour formation), but their growth during the production of fermented foods can lower process costs and increase the adaptation of probiotics leading to enhanced viability. In fermented foods possible antagonisms between starter and probiotic bacteria needs to be checked. Starters can inhibit probiotics but they can also enhance their survival by producing beneficial substances or by lowering the oxygen pressure (Dave and Shah 1997; Kailasapathy and Rybka 1997; Saxelin and others 1999; Saarela and others 2000; Vinderola and others 2002).

The final step in probiotic production process is packaging. The packaging material should be a good oxygen barrier (e.g., aluminum foil) to promote the survival of especially anaerobic probiotic bacteria (bifidobacteria) (Saarela and others 2000). For most probiotic products, transportation and storage at constant refrigerated temperatures is necessary (some dry products may tolerate storage at room temperature). Storage temperature is an important determinant of the shelf-life; with increasing temperatures viability losses can occur rapidly (Saxelin and others 1999).

49.6 PROBIOTIC FOOD MARKET: "HEALTHY FOODS ARE HERE TO STAY"

Probiotic foods have become the fastest growing European food market with an annual growth of over 10% in the EU (Anon 2004). An extensive range of probiotic foods are on the market in different European countries (Table 49.3), most of these being produced by the dairy industry. The application of science-based technology in diet-linked health effects will have a substantial impact in increasing the size of this market. The rapidly moving postgenomic revolution is markedly influencing the nutritional sciences. Key to this is a more thorough understanding of gut microbiota and its interactions with the host and mechanisms contributing to health benefits of pro- and prebiotic intake. Importantly, the European consumer is now increasingly exposed to pro- and prebiotic foods and the public profile for their use is high. The consumer can rightfully demand and expect mechanistic explanations of effects. Further, consumers will expect the pro- and prebiotic foods to be of high quality, which in the case of probiotic foods means that the product has to contain the specific microbe in high enough viable numbers. Surveys reporting sometimes severe quality problems in probiotic products on the market indicate that although probiotic products have gained increasing popularity and their health effects have been assiduously studied, research and development of processes aiming at improving the technological properties of probiotics are still inadequate. Technological improvements are especially important when aiming at diversified application of probiotics in novel and nontraditional products. Technological developments will, however, enable the enhancement of viability of probiotic cultures by, for example, optimizing fermentation and downstream processing conditions, protection of

TABLE 49.3 Examples of Probiotic Food Products on European Market (Information Obtained Mainly from www-pages).

Product Name	Product Type	Manufacturer (Country)	Probiotic Microorganisms in the Products as Stated by the Manufacturer (Information on Product Labels and/or on Web-Pages)*
A-fil	Cultured buttermilk	Arla (Sweden)	*Lactobacilus acidophilus*
A-fil/Lätt A-fil	Cultured buttermilk	Norrmejerier (Sweden)	"Acidophilus bacteria"
A-fil original	Cultured buttermilk	Milko (Sweden)	"Acidophilus bacteria"
Actimel	Dairy drink	Danone (France)	*Lactobacillus casei* immunitass
Aktifit	Dairy drink	Emmi (Switzerland)	*Lactobacillus* LGG
AB-piimä	Cultured buttermilk	Ingman, Valio (Finland)	*L. acidophilus, Bifidobacterium*
Bella Vita	Yogurt drink	Milchhof Meran (Italy)	*L. acidophilus*
Bifisoft	Yogurt type of product	Juustoportti (Finland)	"Bifidobacteria, acidophilus bacteria"
Bifidus	Yogurt drink, yogurt	Emmi (Switzerland)	"Bifidus (and acidophilus bacteria)"
Bighurt	Yogurt	Onken (Germany)	*L. acidophilus, "L. bifidus"*
Biofit (Trink) Jogurt	Yogurt drink, yogurt	Natur pur (Austria)	*L. acidophilus, "Lb. bifidus"*
BiogardePlus family	Yogurt, quark	Almhof (The Netherlands)	*L. acidophilus, L. casei, "Bifidobacterium"*
Biola	Cultured buttermilk, yogurt, yogurt drink	Norwegian Dairies (Norway)	*Lactobacillus* LGG
Biologic Bifidus	Yogurt	Nutrifrais (Switzerland)	*"Bifidobacterium"*
Casilus	Cultured buttermilk	SataMaito (Finland)	*L. casei* 431
Cultura product family	Yogurt drink, fruit drink, cultured buttermilk	Arla Foods (Sweden)	*Lactobacillus casei* F19
Cultura Dofilus	Yogurt	Arla Foods (Sweden)	*Lactobacillus casei* F19, *L. acidophilus, B. lactis*
Dexal pro	Recovery drink	Orion (Finland)	*L. acidophilus, Bifidobacterium*
Dujat Bio Aktiv	Yogurt, yogurt drink	Lura (Slovenia)	*Lactobacillus* LGG
Ekologisk jordgubbs yogurt	Yogurt	Änglamark (Sweden)	*Lactobacillus acidophilus, Bifidobacterium lactis*
Emmifit	Dairy drink	Emmi (Germany), Emmi AG (Portugal)	*Lactobacillus* LGG
Everybody	Yogurt drink	Glanbia Plc. (Ireland)	*Lactobacillus* LGG
Fit & Aktiv	Yogurt, dairy drink	Bauer (Germany)	*Lactobacillus acidophilus* LA5, *Bifidobacterium* BB12
Fjällyoghurt	Yogurt	Milko (Sweden)	*Lactobacillus acidophilus, Bifidobacterium bifidum*
Fundo	Cultured buttermilk	Milka (Finland)	*Lactobacillus rhamnosus* 271

(*Continued*)

TABLE 49.3 *Continued.*

Product Name	Product Type	Manufacturer (Country)	Probiotic Microorganisms in the Products as Stated by the Manufacturer (Information on Product Labels and/or on Web-Pages)*
Fysiq	Yogurt	Campina Melkunie, Mona devision (The Netherlands)	*Lactobacillus acidophilus* Gilliand
Gaio Dofilus	Yogurt	Arlafoods (Sweden/Denmark)	*Lactobacillus acidophilus, Lactobacillus casei* F19, *Bifidobacterium lactis*
Gaio youghurtdryck	Yogurt drink	Arlafoods (Sweden/Denmark)	*Lactobacillus* F19
Gefilac product family	Yogurt, fruit drinks, cultured buttermilks	Valio Sverige Ab (Sweden)	*Lactobacillus* LGG
Gefilus product family	Milk, cultured buttermilk, kefir, yogurt, viili, fruit juice, berry drink, cheese, dairy drink	Valio (Finland, Estonia) RKP Valio (Latvia)	*Lactobacillus* LGG
God hälsa	Ice cream	SIA glass (Sweden)	*Lactobacillus plantarum* 299V
Kaiku Actif	Dairy drink	Iparlat S.A. (Spain)	*Lactobacillus* LGG
Lc1 (go)	Yogurt, dairy drink	Nestle (Germany)	*L. johnsonii*
LGG +	Dairy drink	Mjolkursamsalan (Iceland, Greenland)	*Lactobacillus* LGG
Onaka	Cultured buttermilk	Arlafoods (Sweden/Denmark)	*Bifidobacterium lactis*
Öresundsfil	Cultured buttermilk	Skånemejerier (Sweden)	*Lactobacillus acidophilus, "Bifidobacterium"*
Philura	Cultured buttermilk	Milko (Sweden)	"Acidophilus, bifidus, casei"
Probiotisches Joghurt	Yogurt	NÖM (Germany)	*L. acidophilus*

ProViva product family	Yogurt, berry drink (with oats), recovery drink, dairy drink	Skånemejerier (Sweden)	*Lactobacillus plantarum* 299V
RELA product family	Yogurt, fruit juice, cheese, ice-cream, cultured buttermilk	Ingman (Finland)	*Lactobacillus reuteri*
Verum product family	Yogurt, yogurt drink, cultured buttermilk	NorrMejerier (Sweden)	*Lactococcus lactis* L1A (in yogurt also *Lactobacillus rhamnosus* LB21)
Vifit Vitamel	Yogurt, yogurt drink, fruit drink	Campina Melkunie, Mona devision (The Netherlands)	*Lactobacillus* LGG
ViktVäktarna drickyoghurt	Yogurt drink	Skånemejerier (Sweden)	*Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium*
Vitality	Yogurt, dairy drink	Müller (UK)	*Bifidobacterium* sp., *Lactobacillus acidophilus*
Vivi Vivo	Milk	Granaloro S.p.A.	*Lactobacillus* LGG
Weight watchers	Yogurt	Skånemejerier (Sweden)	*Lactobacillus rhamnosus* 271
Yakult	Dairy drink	Yakult (The Netherlands)	*Lactobacillus casei* Shirota
Yogosan Milbona	Yogurt	No producer information (made for Lidl in Germany)	“Bifidus Bb12, acidophilus”
Yosa	Fermented oat product, oat drink (yogurt -like products)	Bioferme (Finland)	*Bifidobacterium lactis* Bb12, *Lactobacillus acidophilus* LA5

*Invalid or incomplete names are indicated by quotes. Starter microbes are not listed.

cells with carriers (e.g., with probiotics), activation of cells' stress response machineries, and by developing suitable food matrices.

The market pull for functional foods has been so rapid that it has resulted in certain knowledge gaps in the scientific understanding of the mechanisms by which these foods impact health. This has a bearing on the consumer who cannot make an informed choice, the producer who is limited in the reliable development of new and improved products, and authorities that implement new legislation on food claims. This specifically relates to the growing number of probiotic and other functional foods that modulate the human gut. Today, probiotics and prebiotics are marketed successfully since they appeal to the consumer, are bring a positive image and attributes. However, there is a lack of scientific basis for the rational development of probiotic products or for a mechanistic explanation of effects. This prevents further innovation of marketable products that have a clearly demonstrated benefit to the consumer. Gut-related diseases and disorders are very common and the prophylactic management of these problems has a huge market potential. Hence, the current products need to be complemented and an understanding of the scientific basis of their positive attributes to become competitive in the long run and thus meet the demands of European Authorities such as DG Sanco and EFSA, since claims will need mechanistic based evidence of proof in future marketing strategies (Regulation 178/2002/EC, Directives 84/450/EEC, 90/496/EEC, 2000/13/EC, and COM/2003/424).

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50

Enterococci and Dairy Products

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50.1 Introduction 86
50.2 Taxonomy, Physiology, and *Habitat* of Enterococci 86
50.2.1 Taxonomy and Physiology 86
50.2.2 Habitat 88
50.3 Ecology in Dairy Products 88
50.3.1 Presence in Milk and Starter Cultures 88
50.3.2 Presence in Cheeses 89
50.3.3 Presence in Other Dairy Products 90
50.4 Biochemical Properties of Technological Interest 90
50.5 Production of Bacteriocins 91
50.6 Functionality of Enterococci in Dairy Technology 91
50.6.1 Enterococci as Starters 92
50.6.2 Enterococci as Starter Adjuncts 92
50.6.3 Enterococci as Probiotics 92
50.6.4 Enterococci as Protective Cultures 93
50.7 The Other Side of the Story 93
50.7.1 The Antibiotic Resistance 93
50.7.1.1 The Resistance to Glycopeptides 94

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

50.7.2 Presence of Virulence Factors and Genetic Exchange Mechanisms 95
50.7.3 Involvement of Enterococci in Food-borne Illnesses or Food Poisoning 95
50.8 Concluding Remarks 95
References 96

50.1 INTRODUCTION

Bacteria of the genus *Enterococcus* or enterococci (formerly the "fecal" or Lancefield group D streptococci) are ubiquitous microorganisms, but have a predominant habitat in the gastrointestinal tract of humans and animals. Enterococci are not only associated with warm-blooded animals, but they also occur in large numbers in soil, surface waters, vegetables, plant material, and foods, especially those of animal origin such as fermented sausages and cheeses. By intestinal or environmental contamination, enterococci can then colonize raw foods (e.g., milk and meat) and multiply in these materials during fermentation because of their ability to survive adverse environmental conditions such as extreme pH, temperatures, and salinity. Consequently, these bacteria can withstand normal conditions of food production. They also can contaminate finished products during food processing. Therefore, enterococci can become an important part of the microflora of fermented food.

In processed meats, enterococci are generally not desirable because they can cause spoilage. On the contrary, enterococci have important implications in the dairy industry. They play an acknowledged role in the development of organoleptic characteristics during ripening of many traditional cheeses and they have also been suggested as components of cheese starter cultures. Some enterococci of food origin share also a number of useful biotechnological traits such as bacteriocin production and probiotic characteristics.

Unfortunately, enterococci have also recently assumed major importance in clinical microbiology. Enterococci are low grade pathogens but their intrinsic resistance to many antibiotics and their acquisition of resistance to the few antibiotics available for treatment in clinical therapy, such as the glycopeptides, have led to difficulties and a need for new drugs and therapeutic options. Enterococci can cause food intoxication through production of biogenic amines and can be a reservoir for worrying opportunistic infections and for virulence traits, such as production of adhesins and aggregation substances.

50.2 TAXONOMY, PHYSIOLOGY, AND *HABITAT* OF ENTEROCOCCI

50.2.1 Taxonomy and Physiology

The genus *Enterococcus* consists of Gram-positive, oxidase negative, catalase negative, nonsporeforming, ovoid cells arranged in single cells, pairs, or chains. Enterococci are facultative anaerobes with an optimum growth temperature of 35°C. In 1937, Sherman defined them as organisms growing from 10°C to 45°C, in 6.5% NaCl and at a pH of 9.6, surviving heating at 60°C for 30 min, and reacting with Lancefield group D antisera. Since then, besides the original description of *Enterococcus faecium* and *Enterococcus faecalis*, a variety of species have been described. Presently, 31 species are validly published within the genus *Enterococcus*, although recent taxonomic data indicate that two species (i.e., *Enterococcus porcinus* and *Enterococcus villorum*) are synonymous,

whereas *Enterococcus flavescens* is a biovar of *Enterococcus casseliflavus* (Table 50.1; updated September 25, 2004). Newly described species include *Enterococcus canis*, *Enterococcus phoeniculicola*, and *Enterococcus pallens*. However, not all the recently described species meet the physiological and biochemical traits of the typical enterococci (*Enterococcus durans*, *E. faecalis*, *E. faecium*, *Enterococcus gallinarum*, *Enterococcus hirae*, and *Enterococcus mundtii*). There are some species (*Enterococcus cecorum*, *Enterococcus columbae*, *Enterococcus dispar*, *Enterococcus pseudoavium*, *Enterococcus saccharolyticus*, and *Enteroccocus sulfureus*) that do not react with group D antiserum. Other exceptions include *Enterococcus dispar*, *E. sulfureus*, and *Enterococcus malodoratus*, which do not grow at 45°C, and *E. cecorum* and *E. columbae*, which do not grow at 10°C. Although enterococci are generally nonmotile and able to grow in 6.5% NaCl, these traits also seem to create some discordance. For example, *Enterococcus italicus* does

TABLE 50.1 Species Included in the Genus *Enterococcus*.

Species	*Habitat*/Isolation Source
Enterococcus asini	Donkey intestine
Enterococcus avium	Poultry (rare) and mammalian intestines
Enterococcus canis	Dog anal swabs
Enterococcus casseliflavus	Grass, silage, plants, soil
Enterococcus cecorum	Clinical origin, animals
Enterococcus columbae	Pigeon intestine
Enterococcus dispar	Human origin
Enterococcus durans	Clinical isolate
Enterococcus faecalis	Human and other animal intestines
Enterococcus faecium	Human and other animal intestines
Enterococcus flavescens[a]	Clinical origin
Enterococcus gallinarum	Poultry intestine
Enterococcus gilvus	Human clinical specimens
Enterococcus haemoperoxidus	Water/surface waters
Enterococcus hermanniensis	Packaged broiler meat; canine tonsils
Enterococcus hirae	Animal intestines; chicken pathogen
Enterococcus italicus	Italian cheeses
Enterococcus malodoratus	Gouda cheese
Enterococcus moraviensis	Water
Enterococcus mundtii	Grass, silage, plants, soil
Enterococcus pallens	Human clinical specimens
Enterococcus phoeniculicola	Uropygial gland of the Red-billed Woodhoopoe
Enterococcus porcinus[b]	Animal enteric disorders
Enterococcus pseudoavium	Bovine skin
Enterococcus raffinosus	Clinical origin
Enterococcus ratti	Animal enteric disorders
Enterococcus saccharolyticus	Bedding and skin of cattle
Enterococcus seriolicida	Fish pathogen
Enterococcus solitarius	Clinical isolate
Enterococcus sulfureus	Plant material
Enterococcus villorum[b]	Animal enteric disorders

[a]The data of several authors suggest that *Enterococcus flavescens* should be considered an *Enterococcus casseliflavus* biovar rather than a separate species (Descheemaeker and others 1997).

[b]As no phenotypic or genotypic differences were found between these two species, the name *Enterococcus porcinus* is considered to be a junior synonym of *Enterococcus villorum* (de Graef and others 2001).

Source: List of Bacterial Names with Standing in Nomenclature – Genus *Enterococcus* – last update: September 25, 2004. http://www.bacterio.cict.fr/e/enterococcus.html#r.

not tolerate 6.5% NaCl and 0.4% sodium azide. Reliable identification of enterococci to differentiate them both from other Gram-positive, catalase-negative cocci and within the genus often appears difficult. Different media and phenotypical identification schemes have been described and used for routine testing of dairy products. However, given the variability in the biochemical and phenotypic traits of enterococci, molecular based methods are essential for reliable and fast identification, especially from sources with a heterogeneous microflora.

50.2.2 Habitat

The ecology of the genus *Enterococcus* appears greatly diverse (Table 50.1). The main *habitat* of enterococci is the gastrointestinal tract of animals, although the species distribution shows some peculiarities. *E. faecalis* and *E. faecium* are the predominant, Gram-positive cocci in human stools. In production animals like poultry, cattle, and pig., *E. faecium* is the prevalent species but other species such as *E. faecalis*, *E. cecorum*, *E. gallinarum*, and *E. durans* may occur. Some enterococci are also found in the upper and lower human urogenital tracts and in the oral cavity. In addition, *E. casseliflavus* and *E. mundtii* are typical enterococci isolated from plants. Finally, enterococci are also found in water, soil, vegetables, birds, and insects.

50.3 ECOLOGY IN DAIRY PRODUCTS

Many fermented foods made from meat and milk (especially fermented meats and cheeses) contain enterococci. The presence of enterococci in dairy products has long been considered as an indication of insufficient sanitary conditions during the production and processing of milk. On the contrary, their presence in foods has often been shown to be unrelated with direct faecal contamination. Many authors suggested that the presence of certain strains of enterococci may be desirable in some cheeses on the basis of their positive contribution to flavor development during the cheese ripening. This beneficial role has suggested the inclusion of enterococcal strains in starter cultures used in the production of some traditional artisan cheeses.

50.3.1 Presence in Milk and Starter Cultures

Enterococci may enter milk either directly from human or animal faeces or indirectly from contaminated water sources, exterior of the animal and/or from the milking equipment and bulk storage tank. Due to their ability to grow at low temperatures, their heat resistance, and their adaptability to different substrates and growth conditions, enterococci can increase in number during milk refrigeration and survive after pasteurization. Therefore, enterococci are a part of both the raw and pasteurized milk microflora. A variety of enterococci species are found in dairy products but *E. faecalis* and *E. faecium*, and in some cheeses also *E. durans*, remain the species of greatest importance (Giraffa and others 1997; Franz and others 1999, 2003; Giraffa 2003).

Enterococci may occur in natural milk (or whey) starter cultures, which are still widely used for the manufacture of a variety of cheeses, mostly traditional artisan cheeses, produced both in Southern and Northern European countries from raw or pasteurized milk (Giraffa and others 1997). The presence of enterococci in artisan milk starter cultures, which are usually composed of thermophilic lactic acid bacteria, can be explained by their

thermal resistance coupled with their thermophilic nature. In fact, such cultures are still made following the tradition by pasteurising a good quality raw milk and by incubating it at 42–44°C for 12–15 h, thus promoting the natural selection of thermophilic and heat resistant lactic acid bacteria, usually belonging to *Streptococcus thermophilus* and *Enterococcus* spp. Enterococci also are components of the microflora of artisan whey cultures utilized as starters in the manufacture of mozzarella cheese produced by traditional technology from raw water-buffalo milk. The presence of enterococci in these starters is due to their presence in the raw milk, from which the whey is derived, coupled with the mild acidity reached by these cultures after incubation of the whey at the end of cheese-making.

50.3.2 Presence in Cheeses

Enterococci occur as nonstarter lactic acid bacteria (NSLAB) in a variety of cheeses. Their thermal resistance explains their presence in cheeses produced from pasteurized milk exposed to enterococci via natural cheese starter cultures or from adventitious microflora, through post-pasteurization environmental contamination. Consequently, enterococci can be found in cheeses made with both raw and pasteurized milk and their levels depend on the extent of milk contamination, the cheese type, the starter used, and the technology applied, that is, cheese manufacture and ripening conditions. The recovery and persistence of the enterococci during cheese ripening can be attributed to their wide range of growth temperatures, their tolerance to heat, wide pH ranges, and salt.

In some cheeses, especially fresh or soft industrial cheeses made with pasteurized milk and selected lactic starter culture, the presence of enterococci can be deleterious. Some authors have reported that they can cause deterioration in flavor. Their presence, especially at high levels, is the result of a poor hygienic practice during cheese manufacture (Giraffa and others 1997). Therefore, the presence of enterococci in these cheeses is undesirable. Moreover, enterococci are commonly found in high numbers in traditional cheeses produced in Italy, France, Portugal, Spain, and Greece from raw or pasteurized goats', ewes', water buffaloes' or cows' milk. In these cheeses, enterococci can be desirable. Several studies have indicated that strains of enterococci may have a positive influence on the production and ripening of cheeses like Manchego, Armada, Cebreiro, Picante, Majoero, Feta, Teleme, Mozzarella, Monte Veronese, Fontina, Caprino, Serra, Venaco, Comté, and the Irish, Cheddar-like, Baylough cheese. In these cheeses, enterococci comprise a major part of the fresh cheese curd microflora and in some cases they are the predominant microorganisms in the fully ripened product. Levels of enterococci in cheese curd may range from 10^4 to 10^6 CFU/g and in the fully ripened cheese from 10^5 to 10^7 CFU/g. *E. faecium* and *E. faecalis* are the most prevalent species recovered (Franz and others 2003; Giraffa 2003). Table 50.2 summarizes some of the European, artisan cheese varieties in which enterococci have been found.

Since enterococci may dominate the NSLAB microflora of many cheeses, it is supposed that they can positively contribute to the flavor development during cheese ripening. Not only can enterococci influence the flavor and taste of cheeses with their primary and secondary metabolisms, but enterococci also can produce several enzymes that interact with milk components, thus promoting other important biochemical transformations.

TABLE 50.2 Examples of European Cheeses in which Enterococci have been found (Giraffa and others 1997; Franz and others 2003).

Cheese Variety (Country)	Predominant Species in End Product
Manchego (Spain)	*Enterococcus faecium*
Cebreiro (Spain)	*Enterococcus faecalis*
Arzua (Spain)	*Enterococcus faecalis*
Cabrales (Spain)	*Enterococcus faecium*
La Serena (Spain)	*Enterococcus faecium*
Majorero (Spain)	*Enterococcus faecalis*
Picante (Portugal)	*Enterococcus faecium*; *Enterococcus faecalis*
Serra (Portugal)	*Enterococcus faecium*
Feta (Greece)	*Enterococcus faecium*; *Enterococcus faecalis*
Teleme (Greece)	*Enterococcus* spp.
Water-buffalo Mozzarella (Italy)	*Enterococcus faecium*; *Enterococcus faecalis*
Monte Veronese (Italy)	*Enterococcus* spp.
Caprino (Italy)	*Enterococcus faecium*; *Enterococcus faecalis*
Ragusano (Italy)	*Enterococcus* spp.
Pecorino Sardo (Italy)	*Enterococcus faecium*; *Enterococcus faecalis*
Comté (France)	*Enterococcus* spp.
Roquefort (France)	*Enterococcus faecalis*
Beyaz (Turchey)	*Enterococcus* spp.
Baylough (Ireland)	*Enterococcus casseliflavus; Enterococcus faecalis*

50.3.3 Presence in Other Dairy Products

The hazardous presence of enterococci in industrial cheeses, fermented milks and other dairy products (i.e., milk powders) has sometimes been reported. High levels of contaminating enterococci in some cheeses, although not related to a direct fecal contamination, may result from poor hygienic practices during cheese manufacture and lead to deterioration of sensory properties of these products. Apart from some cheeses and artisan starters, therefore, enterococci belong to the spoilage microflora in dairy products.

50.4 BIOCHEMICAL PROPERTIES OF TECHNOLOGICAL INTEREST

The positive influence enterococci may have on cheese seems due to specific biochemical traits such as acidifying, proteolytic, and lipolytic activities, citrate utilization, and production of aromatic volatile compounds. (for extensive reviews, see Giraffa and others 1997; Franz and others 1999, 2003; Sarantinopoulos and others 2001; Giraffa 2003).

In general, enterococci exhibit low milk acidifying ability. The poor acidifying capacity of these microorganisms in milk has been recently reported on strains of dairy origin. *E. faecalis* seems generally a stronger acidifier than *E. faecium*, though its acidifying activity is not comparable with that of other lactic acid bacteria such as *S. thermophilus.*

Proteolysis and lipolysis are the principal reactions responsible for the flavor development in cheeses. Generally enterococci show weak proteolysis, although some strains of *E. faecalis* may have a very strong proteolytic activity. Literature data indicate a marked strain-to-strain variation and no clear relationship has been observed between proteolytic and acidification activities.

The lipolytic activity in enterococci is very variable. Low and often species- or strain-dependent lipolytic activity has been reported. An increase in fatty acids has often been

observed in Cheddar, Feta, Picante, and Cebreiro cheeses. However, only *E. faecalis* showed a degree of lipolysis to permit it to serve as adjunct "lipolytic" starter. The esterolytic system of enterococci is rather complex and more efficient than their lipolytic system. Enterococci show higher activity than strains of most other genera of lactic acid bacteria, with *E. faecium* being the most esterolytic species within enterococci.

Citrate and pyruvate metabolism are important phenotypic traits of many LAB. Citrate in milk is cometabolized by many LAB species into important flavor compounds such as acetate, acetaldehyde, and diacetyl. The ability of enterococci to metabolize pyruvate has been extensively studied, but little is known about their ability to metabolise citrate, in which pyruvate is also an intermediate (Rea and Cogan 2003a,b). Enterococci produce significant amounts of acetate, formate, and ethanol depending on the growth conditions. Pyruvate is the immediate precursor of these products. Furthermore, the breakdown of lactose and citrate during cheese ripening gives rise to a series of volatile compounds, such as acetaldehyde, diacetyl, acetone, and acetoin, which may further contribute to flavor.

Overall, enterococci seem to possess the metabolic potential to actively contribute to the flavor development in fermented dairy products.

50.5 PRODUCTION OF BACTERIOCINS

Bacteriocins produced by LAB are small, ribosomally synthesized, antibacterial peptides or proteins that display a limited inhibitory spectrum against Gram-positive bacteria, particularly closely related strains. Since their inhibitory activity often encompasses food spoilers and foodborne pathogens, they are considered as interesting, natural food biopreservatives. Enterococci are capable of producing a variety of bacteriocins, called enterocins, with activity against *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium* spp., including *Clostridium botulinum* and *Clostridium perfringens*, and *Vibrio cholerae*. Enterocins are small, heat stable nonantibiotics bacteriocins with a generally strong antilisterial effect. Such properties meet some of the characteristics required for compounds to be used as antimicrobials in food products (Giraffa 1995, 2003; Franz and others 1999, 2003).

Considerable research has been carried out on characteristics of enterocins, or enterocin-producing strains, for a potential application in dairy technology. Dairy-grade traits of enterocins often have been reported, such as production and stability in milk in the temperature range 30–37°C, insensitivity to rennet and heat, a general compatibility with starter LAB species, and stability over a wide range of pH values. It also has been observed that enterocins may lyse starter cells, thus potentially increasing the bacterial enzyme pool in the cheese curd and accelerating cheese ripening and flavor production. Therefore, the application of enterocins, either as a food additive or through in situ production by an appropriate starter culture or co-culture, for the manufacture of cheese, seems promising.

50.6 FUNCTIONALITY OF ENTEROCOCCI IN DAIRY TECHNOLOGY

As stated above, enterococci usually represent an important part of the bacterial flora of many ripened cheeses. Therefore, their possible role and functionality in dairy fermented products has been thoroughly studied. Examples of the most important applications of enterococci in dairy products are given below (for a review, see Giraffa 2003).

50.6.1 Enterococci as Starters

Two important observations have encouraged the application of enterococci as starters:

- Presence of strains with good technological properties within *E. faecium* and *E. faecalis* isolated from cheeses;
- Frequent isolation of enterococci from milk starter cultures used for the manufacture of Italian artisan cheeses.

However, from the early works on Cheddar cheese no significant research reports followed. The generally low acidifying and proteolytic activities of enterococci limits their application as starter cultures for cheese production.

50.6.2 Enterococci as Starter Adjuncts

Starter adjuncts are defined as cultures added to cheese for purposes other than acid formation, which is mostly devoted to starter activity. Starter adjuncts can be added to accelerate ripening, produce desirable flavor, or act as probiotics. Several research works have been carried out to evaluate the feasibility of selected enterococci to act as starter adjuncts in cheese production. *E. faecium*, *E. faecalis*, and *E. durans* have been proposed in combination with both mesophilic and thermophilic LAB species as a part of defined starter cultures for different European cheeses such as Italian semicooked cheeses, water-buffalo Mozzarella, Venaco, Cebreiro, and Hispanico. Generally, the presence of the added enterococcal flora throughout ripening positively affects taste, aroma, color, and structure, as well as the overall sensory profile, of the full-ripened cheeses. This seems linked to the increased amount of soluble nitrogen, total free amino acids, volatile free fatty acids, long-chain free fatty acids, and diacetyl and acetoin in cheeses made with enterococci.

Many European cheeses are characterized by complex bacterial surface flora, which generally consists of yeasts, coryneform bacteria, and micrococci or coagulase-negative staphylococci. However, also enterococci can often be found as nondominant, surface bacteria. Within bacterial surface ripening flora of French and German smeared cheeses, different *Enterococcus* spp. strains colonizing the cheese rind are able to inhibit *Listeria.* Therefore, enterococci can be promising candidates for the development of a defined surface-smear ripening flora.

50.6.3 Enterococci as Probiotics

Enterococci possess properties that allow them to be used as probiotics. To this regard, the Advisory Committee on Novel Foods and Processes decided to allow the use of *E. faecium* strain K77D as a starter culture in fermented dairy products (ACNFP 1996). It is generally known that products for human consumption containing probiotic organisms may be grouped into three categories: infant foods, cultured milks, and pharmaceutical preparations. However, a relatively new frontier in the probiotic technology is the use of cheeses as delivery systems of probiotic strains used as functional starters. A strain of *E. faecium* has been used as probiotic adjunct culture in Cheddar cheese. This strain has a number of properties required of a probiotic microorganism, including the ability to relieve irritable bowel syndrome, intestinal origin, no pathogenicity, and tolerance to

bile and acid (therapeutic adjuncts). Compared with the control, increased proteolysis and higher levels of odor-active volatile compounds were observed in cheese containing the *E. faecium* adjunct throughout the ripening period. The strain maintained viability in Cheddar cheese during nine to 15 months of ripening at 8°C.

50.6.4 Enterococci as Protective Cultures

Bacteriocin-producing strains, defined as "protective cultures" when applied to food, belong to a particular class of starter adjuncts. Several studies in milk, soft cheeses, and soy milk demonstrate the inhibitory effect of enterocin-producing *E. faecium* and *E. faecalis* against *L. monocytogenes* and *S. aureus*. The presence and the anti-*Listeria* activity of enterocins produced by protective cultures in cheese persist throughout the ripening process. Generally, enterocins have little effect on both the commercial starter activity and the organoleptic characteristics of the products. In some cases, the complex curd (or cheese) environment may interfere with bacteriocin production levels. Alternatively, the lack of growth of the enterocin-producing strains, may affect the in situ bacteriocin efficiency.

The functionality of enterocin-producing strains under industrial, cheese processing conditions seems, therefore, strongly dependent on the cheese system and the technology applied.

50.7 THE OTHER SIDE OF THE STORY

Over the last two decades, enterococci, formerly viewed as organisms of minimal clinical impact, have emerged as important hospital-acquired pathogens in immunosuppressed patients and intensive care units. The newly accentuated ambiguity concerning the relationships of these bacteria with human beings is related to their enteric *habitat*, their entering the food chain, their possible involvement in foodborne illnesses due to the presence of virulence factors, such as production of adhesins and aggregation substances, and their antibiotic resistance (for a review, see Giraffa 2002).

The clinical significance of enterococci in human infections is poorly understood because of the scarcity of well-documented reports confirming their occurrence in mixed cultures. Table 50.3 summarizes hospital acquired infections in which enterococci have been somehow implicated (Moellering 2000; Kayser 2003).

50.7.1 The Antibiotic Resistance

Development of antibiotic resistance in bacteria is mainly based on two factors, the presence of resistance genes and the selective pressure by the use of antibiotics. Antibiotic resistance, which can be both intrinsic and acquired, makes enterococci effective opportunists in nosocomial infections. The extremely high level of intrinsic antibiotic resistance within enterococci, coupled with the selective pressure imposed by the use of antibiotics both in clinical therapy and animal husbandry, led to increased selection of resistant strains. The widespread finding of these microorganisms in raw foods could be the key factor contributing to the spreading of antibiotic resistant enterococci (ARE) in both unfermented and fermented foods. ARE have been found in meat products, dairy products, ready-to-eat foods, and even within enterococcal strains proposed as probiotics (Giraffa 2002). In this regard, resistance to the glycopeptides

TABLE 50.3 Summary of the Most Frequent Infections Caused by Enterococci (Moellering 2000; Kayser 2003).

Pathology[a]	Remarks
Urinary tract infections	Often associated with catheterisation in hospitalised patients
Bacteremia	Enterococci are observed in approx. 6–7% of all bacteria isolated from the blood stream. Portal of entry: urinary tract infections; intra-abdominal infections; burn wounds; diabetic foot infections; intravascular catheters
Intra-abdominal and pelvic infections	Enterococci are found as part of a mixed flora
Wound and tissue infections	Enterococci are found as part of a mixed flora
Endocarditis	Enterococci are observed in approx. 5–15% of all cases. Common sources: genitourinary and biliary portals
Rare enterococcal infections	Meningitis; respiratory tract infections

[a]In patients with severe underlying diseases.

vancomycin and teicoplanin is the widest known example of antibiotic resistance of enterococci.

50.7.1.1 The Resistance to Glycopeptides. The selection and spreading of enterococci resistant to the glycopeptide antibiotics vancomycin and teicoplanin have become a clinically significant emergence in the hospital environment (for reviews, see Giraffa 2002; Klein 2003). The use of this class of antimicrobials is of utmost importance in clinical therapy against multiple antibiotic-resistant strains, especially for patients allergic to other antibiotics, for example, ampicillin and penicillin. Although nosocomial acquisition and subsequent colonization of vancomycin-resistant enterococci (VRE) has been emphasized among hospitalized persons, colonization appears to occur frequently in persons not associated with the healthcare setting. Several studies conducted in European countries and the United States in recent years indicate that colonization with VRE frequently occurs in the community and that many animal, food, and environmental reservoirs can act as sources for VRE outside the health care setting. In this mechanism, the transport of these resistances via the food chain to humans seems likely to occur and dairy products could act as vectors. From literature data, however, the presence of VRE in cheeses is still controversial and not so frequent as reported for meat products.

An association has been suggested among the use of avoparcin and other antibiotics in livestock, the selection of VRE, and bacterial colonization of humans via the food chain. This led to a European ban for use of avoparcin (by 1997) and other growth promoters in animal feeds.

The increasing resistance of enterococci to antibiotics is exacerbating the increased occurrence of these bacteria as nosocomial opportunists. Additionally, the finding of resistant strains outside the nosocomial environment widens the risk of human exposure to opportunistic pathogens. Those at greatest risk include the elderly and children who, for opposite reasons, may have deficient immune status. However, the antibiotic resistance alone cannot explain the virulence of these bacteria in the absence of pathogenicity factors and active mechanisms of gene transfer.

50.7.2 Presence of Virulence Factors and Genetic Exchange Mechanisms

Virulence traits in enterococci include adherence to host tissue, invasion and abscess formation, resistance to, and modulation of, host defense mechanisms, secretion of cytolysins and other toxic products and production of plasmid-encoded pheromones. A number of genes encoding for virulence factors in enterococci have been sequenced and characterized and their effects have been shown in human and animal studies. The incidence of virulence determinants among food isolates studied so far appears to be strain-dependent. Furthermore, it may be argued that strains which possess multiple virulence determinants associated with various phases of infection may pose a higher risk than strains which possess a single virulence trait (Franz and others 2001).

It has recently been suggested that enterococcal disease is a two-step process. Colonization of the gastrointestinal tract by strains carrying virulence determinants and/or antibiotic resistance is followed by population spreading. It could be deduced, therefore, that strains lacking virulence and antibiotic-resistance determinants introduced into the human gut via dairy products would not be of risk for immunocompetent individuals. A well-documented example is *E. faecium* strain SF68, used in pharmaceutical preparations (Franz and others 1999).

Many of these enterococcal virulence traits, such as haemolysin-cytolysin production, adhesion ability, and antibiotic-resistance, have been shown to be transmissible by gene transfers mechanisms. Trans-conjugation in which starter strains acquire virulence determinants from clinical-associated strains also has been demonstrated (Giraffa 2002).

50.7.3 Involvement of Enterococci in Food-borne Illnesses or Food Poisoning

High levels of biogenic amines in many fermented foods, such as fermented sausages, cheeses, wines, fermented olives, and fish products, involved in food intoxication, may be a clinical concern. Food intoxication caused by ingestion of biogenic amines determines a number of symptoms of increasing complexity that include headache, vomiting, increased blood pressure, and allergic reactions even of strong intensity.

Microbial agents involved in biogenic amine production in foods may be derived from either starter and nonstarter LAB or contaminating microflora. Cheeses may represent a good substrate for production and accumulation of biogenic amines, especially tyramine, from enterococci able to decarboxylate free amino acids into the matrix. The ability to produce biogenic amines in cheese and fermented sausages has been reported for bacteria of the genus *Enterococcus* (Giraffa and others 1997; Giraffa 2002).

50.8 CONCLUDING REMARKS

There is no consensus on the acceptance of enterococci in foodstuffs and their role as primary pathogens is still a question mark. On one hand, there is positive evidence that enterococci can be useful in dairy technology. Some well defined strains with a long

history of safe use as probiotics are known. In addition, enterococci could be beneficial, as starter adjuncts, for the biopreservation or improvement of organoleptic characteristics of different cheeses. On the other hand, the emergence of enterococci resistant to glycopeptides and other antibiotics, the production of biogenic amines in cheese, and the finding of virulence traits within both clinical and foodborne isolates raise questions about the safety of enterococci in foods. It has been suggested that application of enterococci in cheeses could proceed once pathogenic and nonpathogenic strains are defined on the basis of careful selection and case-by-case studies. However, the finding of effective gene transfer mechanisms within enterococci weakens this statement. Clearly, the barrier separating enterococci as commensals (and/or opportunistic pathogens) from pathogens appears most fragile.

It is expected, therefore, that cheeses (especially those made with raw milk) could contain enterococci with virulence traits and resistances to clinically important antibiotics, due to the occurrence of direct environmental contamination. Therefore, while some time ago enterococci were put forward as co-cultures in food, now scientists are aware of the possible health hazard enterococci can pose. Teuber and others (1999) recommended as a drastic measure that cheese should be produced only with pasteurized or microfiltered milk. That means starter cultures should be used in cheese manufacturing. But in many artisan European cheeses, producers still establish the quality of the product on the use of raw milk, often without starter addition. On the other hand, in the Directive 2000/54/CE of the European Parliament, concerning risks of exposure to biological agents, *Enterococcus* spp. are allocated into risk-group 2, which includes microorganisms harboring potential virulence factors. Therefore, more research efforts on clinical and epidemiological techniques to discriminate between pathogenic and nonpathogenic *Enterocccus* spp. are needed. Moreover, the still unclear taxonomy of enterococci will demand molecular tools to achieve more effective identification and characterization of natural isolates in epidemiological and clinical studies. A grouping of the strains on the basis of virulence traits, as well as case-by-case studies on overall phenotypic and genotypic characteristics, could enable the development of pathogenicity schemes within the enterococcal population to better comprehend the microbial ecology of this heterogeneous group of bacteria.

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51

Fermented Milk: Health Benefits Beyond Probiotic Effect

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51.1 Introduction 99
51.2 Traditional Health Claims Associated with Fermented Milk 100
51.3 Production of Fermented Milk 101
51.4 Regulatory Aspect Pertaining Fermented Milk 103
51.5 Nutritional and Physiological Benefits of Fermented Milk 105
51.6 Health Benefits of Fermented Milk 106
51.6.1 Alleviation of Lactose Intolerance 106
51.6.2 Antiosteoporotic and Antiobesity Effect 107
51.6.3 Physiological Activity 108
51.7 Future Trends 110
References 111

51.1 INTRODUCTION

The fermentation of foods, especially of dairy origin, presents one of the oldest methods of food preservation known to mankind. The origin of fermented milk was placed in the Middle East before the Phoenician era. As early as 7000 BC, the Egyptian consumed their, now traditional, fermented milk products, Laban Rayeb and Laban Khad. Persian

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tradition claims that Abraham owed his longevity to consumption of sour milk (Kosikowski and Mistry 1997). Similarly, peasants of Thrace enjoyed benefits of "Prokish", made by souring of sheep milk. Traditional production of fermented milk established in the middle and Far East was spread throughout the east Europe and Russia by the Tartars, Huns, and Mongols during their invasions. Consequently, a wide range of fermented dairy products still exists in these regions and some popular products such as yogurt and kefir are claimed to originate from the Balkans and Eastern Europe. Although our knowledge has expanded tremendously in recent decades shading some light on the role of microbial agents involved in the fermentation processes, initially traditional fermentation approaches were artisinal in nature without any appreciation for the role of microorganisms. Nonetheless, continuous observations and experience resulted in establishment of certain procedures by which handling and storage of milk in a certain manner would produce products than not only had a very good keeping quality but also pleasant sensory characteristics. By the middle of the 19th century, the industrial revolution resulted in the concentration of large masses of population in cities requiring a manufacturing of products in large quantities. Clearly, the natural fermentation and traditional manufacturing approach such as back-slopping were inappropriate bases for any large-scale industrial process. By the end of the 19th century, the microorganisms involved in dairy fermentations were isolated and characterized leading to a large scale production of defined starter cultures.

51.2 TRADITIONAL HEALTH CLAIMS ASSOCIATED WITH FERMENTED MILK

Although the preservative role of fermented dairy products was appreciated early, it was not until the late 19th century that scientists realized that a wide range of traditional sour milk might have had additional benefits in addition to prolonged shelf-life and pleasant sensory properties. At the beginning of the 20th century, Nobel Laureate Ilya Metchnikoff (Metchnikoff 2004) noticed that Bulgarian peasants had an average lifespan of 87 years, exceptional for the early 1900s, and that four out of every thousand lived past 100 years of age. One of the major differences in their lifestyle in comparison to the contemporary diet was large consumption of fermented milk. In his well known autointoxication theory, Metchnikoff suggested that the human body was slowly poisoned by toxins present in the diet, and body's resistance steadily weakened by proliferation of enteric pathogens, all of which successfully prevented by consumption of milk and lactic acid producing bacteria. In the early 1900s, his statement resulted in a surge in the consumption of fermented milk. The bacterium responsible for this fermentation was termed *Bacillus* and was believed to have a substantial positive effect on health. However, in the 1920s, it was demonstrated that this bacterium, later called *Lactobacillus bulgaricus*, could not survive the passage through the gastrointestinal tract, which disputed Metchnikoff's theory. Nonetheless, the scientists continued to investigate possible benefits of bacteria to the health. In the 1930s, certain strains of *Lactobacillus acidophilus* were isolated and found to be capable of colonizing the human digestive tract where they exerted appreciable physiological activity. These findings led to the production of a fermented dairy product in which a single strain of *L. acidophilus* was used in the fermentation (Burke 1938). Since that time, a number of fermented milk products containing live microbial flora has been introduced on the market supported by an extensive research that lead to introduction of probiotic concept (Fuller 1992).

Another astonishing example of the effect of diet on the longevity is seen in the Hunza Valley, located between the borders of China, India, Pakistan, and Russia at nearly 2500 m. The Hunzakuts, inhabitants of this valley, have been attracting the attention of the scientific community because of their excellent health and extremely long lifespan (Hoffman 1968). Similarly, Abkhasians and Georgians, from an area that used to be known as part of the Soviet Union, have been highlighted by their amazing history of longevity. The absence of diseases, active lifestyle and sound basic diet have resulted in people living to be 100, 110, or 120, and occasionally as much as 140 years of age. Importantly, a substantial part of the diet (one to two glasses per day) is composed of fermented milk (Benet 1974). The Georgians strongly believe that health depends very much on consumption on fermented milk. A known Georgian saying claims that the longevity comes with consumption of sour milk.

51.3 PRODUCTION OF FERMENTED MILK

Food fermentation has been carried out for centuries in an attempt to long-term preserve perishable foods such as milk. As a consequence, a great variety of fermented dairy products has been developed, each with distinct sensory characteristics. The fermentation process in general involves the fermentation of an organic material, mainly carbohydrates, resulting in a range of products, which include principally organic acids, alcohol, and carbon dioxide (de Vos 1996). These metabolites proved a preservative effect by inhibiting the growth of spoilage and/or pathogenic microflora in fermented products. Additionally, a number of other metabolites may be produced that would affect the quality of the final fermented milk including the flavor compounds such as diacetyl and acetaldehyde, as well as compounds that may have positive health implications such as vitamins, antioxidants, and bioactive peptides. When considering dairy fermentations, a bacterial group commonly referred to the lactic acid bacteria (LAB) is primarily responsible for majority of the microbial transformations. This group is composed of a number of genera including *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Leuconostoc*, and *Pediococcus*. They are strictly fermentative, producing lactic acid as the main end-product. They also lack the ability to synthesize haeme thus they are consequently catalase-negative and lack a terminal electron transport chain (Stiles and Holzapfel 1997). Members of the LAB are usually subdivided into two distinct groups based on their carbohydrate metabolism. The homofermentative group consisting of *Lactococococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus*, and some lactobacilli utilize the Embden–Meyerhof–Parnas (glycolytic) pathway to transform a carbon source chiefly into lactic acid. By this pathway, 1 mol of glucose will be converted into 2 mol of lactate with subsequent generation of 2 mol of ATP. As opposed to homofermentors, heterofermentative bacteria produce equimolar amounts of lactate, CO_2, ethanol, or acetate from glucose exploiting phosphoketolase pathway. The utilization of carbohydrates by this route generates only half the energy of the homofermentative group. Members of this group include *Leuconostoc*, *Weissella*, and some lactobacilli. The species belonging to *Enterococcus* genus are frequently found in traditional fermentations and may be included as a component of some mixed starters. However, their deliberate utilization in dairy fermentations still remains controversial, especially since some of the species have been now recognized as opportunistic human pathogens associated with hospital-acquired and urinary tract infections (Franz and others 1999).

Dairy LAB are fastidious in nature requiring a wide range of essential growth promoters. Although considered a rich growth medium, milk contains small amounts of peptides and free amino acids to efficiently support growth of LAB (Vasiljevic and others 2005). In response to this limitation, LAB have developed a complex proteolytic system consisting of proteinases and peptidases, which enable them to utilize casein as an additional source of amino acids and nitrogen (Smid and others 1991). The structural components of the proteolytic systems of LAB can be divided into three groups on the basis of their function including proteinases (that break down caseins to peptides), peptidases (that degrade peptides), and transport systems (that translocate the breakdown products across the cytoplasmic membrane) (Kunji and others 1996). The first step in casein degradation is mediated by cell wall-located proteases, which cleave caseins to oligopeptides. Further degradation to smaller peptides and amino acids that can pass through the cell membrane is performed by peptidases (Shihata and Shah 2000). Furthermore, dairy LAB grow rapidly at ambient temperature, converting lactose to lactic acid. Consequently, pH of milk is reduced and, around pH 4.6, the isoelectric point of caseins is reached, at which a gel is formed as one of the main sensory detriments of the fermented dairy products. A wide range of fermented milk has been developed since the first documented production and some 400 generic names have been applied to the traditional and industrialized fermented milk throughout the world (Kurmann and others 1992). However, most of these products are basically very similar and very accurate description would include only several varieties. In this paper, we aimed to classify all these products taking into

TABLE 51.1 Some Commercially Available Fermented Milk Products and Cultures Involved in the Fermentation (Some Details Compiled from Heller 2001; Kosikowski and Mistry 1997; Pato and others 2004; Tamime and Marshall 1997).

Type of Fermentation	Product	Culture
Lactic–mesophilic	Taetmojolk	*Lactococcus lactis* ssp. *lactis*
	Folkjolk	*Lc. lactis* ssp. *lactis* biovar. *diacetylactis, Leuconostoc mesenteroides* ssp. *cremoris*
	Ymer	*Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis*
	Cultured buttermilk	*Lc. lactis* ssp. *cremoris, Leuc. mesenteroides* ssp. *cremoris, Lc. lactis* ssp. *lactis* biovar. *diacetylactis*
	Evolus®	*Lactobacillus helveticus*
Lactic–thermophilic	Yogurt	*Lb. delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus*
	Bulgarian buttermilk	*Lb. delbrueckii* ssp. *bulgaricus*
	Dahi	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus*
	Zabadi	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus*
Yeast–lactic	Kefir	*Lc. lactis, Lc. lactis* ssp. *cremoris*, *Leuc. mesenteroides*, *Leuc. mesenteroides* ssp. *dextranicum*, *Lb. kefir, Lb. fermentum, Lb. brevis, Lb. reuteri, Acetobacter pasterurianus, Candida kefir, Saccharomyces cerevisiae, Kluyveromyces marxianus*
	Kumys	*Lb. delbrueckii* ssp. *bulgaricus, Lb. acidophilus, Saccharomyces lactis*
	Calpis®	*Lb. helveticus, Saccharomyces cerevisiae*
	Dadih	*Lc. lactis* ssp. *lactis, Leuc. paramesenteroides,* yeast
Mold–lactic	Villi, piimi	*Lc. lactis* ssp. *cremoris*, *Geotrichum candidum*

account the type of milk used, the dominating microbial flora and principal metabolic products (Tamime and Marshall 1997). The proposed scheme divided fermented milk into three broad categories: (1) lactic fermentations, (2) yeast-lactic fermentations, and (3) mold-lactic fermentations. Some products based on this classification are listed in Table 51.1 with yogurt being the most important type.

51.4 REGULATORY ASPECT PERTAINING FERMENTED MILK

The existing legislation regulating production of fermented milk in many countries classifies the product based on the chemical composition or fat content (full, semiskimmed, or medium; and skimmed or low). According to general standards published by the International Dairy Federation (IDF 1992a,b), fermented milk could be defined as product prepared from milk and/or milk derived components, produced from raw materials that were at least pasteurized by the action of specific microflora, which results in a pH reduction and subsequent casein coagulation. Additional IDF specifications recommend that (1) the starter cultures be viable, active and abundant having concentration of at least 10^7 colony forming units (cfu) per g of a product throughout the shelf-life; (2) raw materials used in production must be at least pasteurized, optionally homogenized and addition of certain additives such as thickeners is rather optional; (3) final products should have a shelf-life of up to 30 days stored at 4–7°C; (4) as the presence of live culture is required at the time of sale, heat-treatment to prolong the storage stability be not permitted; and (5) syneresis (wheying off) in the final product needed to be avoided with the exception of concentrated fermented milk products such as labneh and ymer.

The properties of the final product are substantially affected by the culture used for the fermentation, thus the identity of the starter cultures is well defined. Traditionally, yogurt is the coagulated milk produced by lactic acid fermentation using a mixed starter culture consisting of thermophilic strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. In some countries, a provision has been made to include other species of LAB. Consequently, some yogurt manufacturers use *Lactobacillus helveticus* and *Lactobacillus jugurti* during production. Similarly, yogurt can be made using an ABT starter cultures composed of *S. thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* spp. as practiced in Australia. The fermentation greatly depends on the rapid acid production by *S. thermophilus*. On the other hand, the U.S. standard prohibits the use of any other starter culture but *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* in yogurt-making (Shah 2003).

The fermented milk can also be produced by mesophilic starter cultures giving products that are generally included under lactic and yeast–lactic fermentations. The mesophilic LAB belong to the genera of *Lactococcus* (*Lac. lactis* ssp. *lactis*, *Lac. lactis* ssp. *lactis* biovar *diacetylactis*, and *Lac. lactis* ssp. *cremoris*) and *Leuconostoc* (*Leu. mesenteroides* ssp. *cremoris* and *Leu. mesenteroides* ssp. *dextranicum*). These organisms are usually used in the production of fermented milk as defined, mixed cultures. On the other hand, products such as kefir and kumys (kumiss, koumiss) are made by yeast–lactic fermentations utilizing the ability of mixed starter cultures that are rather complex consisting of numerous species of LAB, yeasts, and molds.

Kefir is a refreshing drink originating from the northern slopes of the Caucasian mountain range. Artisinal production included addition of starter culture in the form of "kefir grains" to bovine or caprine milk and prolonged storage in leather sacks at different

environmental temperatures–during the day exposed to the sunlight and at night protected from the overly low temperature (Koroleva 1991). The mixture was frequently shaken to allow for an even distribution of metabolites as well as to enhance the fermentation. The final product usually contained substantial amount of lactic acid, ethanol, and carbon dioxide. The continuous production was achieved by either back-slopping, adding fresh milk to small quantities of kefir, or sieving off of kefir grains that would subsequently be reused in the production of new batches (IDF 1984). Kefir grains are composed of a complex microbial mixture consisting of mesophilic, homofermentative lactococci, usually *Lac. lactis* ssp. *lactis* and *Lac. lactis* ssp. *cremoris*; obligate heterofermentative lactobacilli such as *L. kefir*; mesophilic heterofermentative leuconostoc; acetic bacteria such as *Acetobacter* and yeasts such as *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, and *Candida kefir* (Koroleva 1991). The presence of each of these groups results in noticeable effect on properties of the final product.

Kumys is another fermented milk product produced by a mixed yeast–lactic fermentation of mare's milk. Similar to kefir, kumys has a long tradition dating some 25 centuries back when the Scythian tribes enjoyed benefits of this refreshing drink (Koroleva 1991). Traditionally, fresh mare milk was filled into leather sacks and agitated with a wooden paddle. The mixed culture involved in the fermentation consisted of several types of yeasts, such as *Saccharomyces lactis* and *Sac. cartilaginosus*, and thermophilic strains of *L. delbrueckii* ssp. *bulgaricus* and mesophilic strains of *L. causasicum* (Koroleva 1991). Nowadays, modern manufacturing of kumys includes a controlled fermentation of bovine skim milk by defined starter cultures. Another example of a product produced through mixed yeast–lactic fermentation comes in the form of "dadih," an Indonesian fermented milk. Traditionally it was produced by filling a bamboo tube with fresh buffalo milk; the tube was closed with banana leaves and fermentation performed at room temperature for about two days. The LAB cultures involved in fermentation included *Leuconostoc* sp. and *Lactobacillus* sp. and several genera of yeasts (Surono 2003).

Simultaneous growth of yeasts and LAB in fermented dairy products leads to possible interactions that may affect final product characteristics and quality. The substantial amounts of carbon dioxide and ethanol are produced due to presence of yeasts imparting desirable characteristics to products such as kefir and kumys. Co-existence may result in either stimulation or suppression of growth of the co-cultured strains. Competition for essential nutrient or the production of growth factors may play a role in any of these occurrences (Gadaga and others 2001). Importantly these interactions occur during very short period of time, usually during main fermentation that takes two to three days.

Final fermented products are consumed immediately after production or stored at low temperature that halts the growth of the cultures. During this short fermentation time, a range of compounds may be produced that would influence flavor and/or the consistency of the product due to the formation of an acid gel, production of extracellular polysaccharide and carbon dioxide. In some instances, the growth appears to be symbiotic, where a compound produced by one organism is utilized by another. Mesophilic strains of *Leuconostoc* are capable of metabolizing acetaldehyde produced by lactococci strains (Tamime and Marshall 1997). The main product of LAB metabolism is lactic acid; the presence of this in milk results in a gel formation as well as imparts a sour taste to the product. Citrate metabolism plays an important role resulting in formation of various flavor compounds such as diacetyl, acetic acid, and carbon dioxide (Hugenhotlz 1993). The proteolytic activity of LAB releases a range of peptides and free amino acid that serve as precursors of numerous flavor compounds, that is, malty flavor is imparted by branched

chain amino acids through the metabolism of several strains belonging to *Lactococcus* and *Lactobacillus* genera (Ayad and others 1999). Also acetaldehyde, the characteristic flavor compound in yogurt, is produced from threonine.

51.5 NUTRITIONAL AND PHYSIOLOGICAL BENEFITS OF FERMENTED MILK

Considering their nutritional profile, fermented dairy products resemble a dairy base that they are made of, most frequently full-fat or skim milk. The natural function of milk is to provide complete nutritional requirements to the neonatal mammal. The composition of milk is a species dependant resulting in great interspecies differences (Fox 2003). Although very similar in the total solids content, major differences between human and bovine milk are found in protein, 1–3.4%, respectively, and lactose content, 7–4.8%, respectively (Fox and McSweeney 1998). Bovine milk is composed of well-balanced micronutrients including carbohydrate, fat, and protein. It contains on average 4.8% lactose, 3.4% protein, 3.7% fat, and 0.7% ash. Milk proteins, in general grouped into two classes: caseins and whey proteins, have high nutritive value due to favorable balance of essential amino acids (Buttriss 1997). In addition to macronutrients, milk contains a range of bioavailable micronutrients such as calcium, phosphorus, riboflavin (vitamin B_2), thiamin (vitamin B_1), and vitamin B_{12}, as well as folate, niacin, magnesium, and zinc.

In addition to exceptional nutritional attributes, milk and milk-derived products such as fermented milk contain components that possess a range of different bioactivities, some of them summarized in Table 51.2. Consequently, some of the milk native and derived constituents are being frequently considered functional, thus making dairy products an important part of functional foods and nutraceuticals. Although the concesus regarding an appropriate definition of functional foods has not been reached yet, many attempts have described them as foods similar in appearance to a conventional food, consumed as a part of regular diet, and have shown a particular physiological benefit and/or demonstrated a potential in reducing the risk of chronic disease beyond basic nutritional functions (Lucchina 2003).

TABLE 51.2 Biogenic Activity of Native Milk Macro-components (Compiled from Pariza 2004; Schmelz and others 2000; Shah 2000).

Component	Form	Bioactivity
Protein	Caseins	Mineral carriers, antiosteoporotic, precursor of bioactive peptides
	α-lactalbumin	Modulation of lactose metabolism, Ca carrier, immunomodulation
	β-lactoglobulin	Retinol carrier, fatty acid binder, presumed antioxidative activity
	Immunoglobulins	Immune activity
	Lactoferrin	Antimicrobial, antioxidative, immunomodulation, anticarcinogenic
	Lactoperoxidase	Antimicrobial
	Lysozyme	Antimicrobial
Fat	Conjugated linoleic acid	Anticarcinogenic, modulation of lipid and protein metabolism, antiinflamatory, hypotensive, antiatherosclerotic
	Sphingolipids	Anti-inflamatory, anticarcinogenic
	Butyric acid	Anticarcinogenic
Carbohydrates	Oligosaccharides	Prebiotic, antimicrobial (antiadhesive), Ca absorption

Milk proteins exert an appreciable range of different physiological activities in their native form. Specific immunoglobulins provide the first line of defense to suckling neonates through passively acquired immunity. Other nonspecific antimicrobial milk factors such as iron-binding protein, lactoferrin, and several enzymes including lactoperoxidase and lysozyme are more actively involved in preventing a microbial proliferation (Floris and others 2003). More importantly, the functionality of dairy proteins is further enhanced upon liberation of bioactive peptides by proteolysis (Gobbetti and others 2002). Proteolysis is performed by naturally occurring enzymes in milk, bacterial from starter cultures as well as enzymes of the digestive tract, resulting in release of a great deal of peptides with different biogenic activities such as opioid, hypotensive, immunomodulating, antithrombotic, and antimicrobial activities.

Milk also contains a quantity of complex lipids, particularly phospholipids and sphingolipids. Their excessive concentration is a result of milk fat globule formation during which the globules are encapsulated in an entire layer of epithelial cell plasma membrane. A variety of sphingolipids showed a substantial influence on cell proliferation. They were very successful in reducing a number of neoplatic cells during early stages of cancer development (Schmelz and others 2000). The key factor determining the efficiency of cancer suppression appears to be a product of sphingolipids hydrolysis, ceramides.

In 1979, Pariza and others (1979) reported that grilled ground beef contained both bacterial mutagens and a substance that inhibited mutagenesis. The group later went on to identify the new anticarcinogen as conjugated linoleic acid (CLA) (Ha and others 1987). This discovery led to further investigations, not only on the establishment of the underlying mechanism(s) for the prevention of carcinogenesis but also identifying other potential applications. The potential benefits are numerous ranging from anticancer activity, immune enhancement and antiinflammatory activity over to obesity and weight control as well as antiatherosclerotic effect (Pariza 2004). Initially it was believed that all these effects were expressed by a single compound. However, CLA is a term describing a class of dieonic isomers of linoleic acid, with several of them possessing biological activity. So far, only two isomers have been identified to have known physiological effects: *c*9,*t*11-CLA and *t*10,*c*12-CLA (Pariza 2004). CLA is produced as a metabolic intermediate from polyunsaturated fatty acids, specifically linoleic (18 : 2n-6) and linolenic acids (18 : 3n-3), by rumen bacteria, namely *Butyrivibrio fibrisolvens* and *Megasphaera elsdenii* (Kramer and others 2004). Consequently, the richest sources of CLA are products of animal origin with milk and yogurt containing between 0.46–1.78 and 0.43–1.12 g per 100 g of total fatty acids, respectively (Gnädig and others 2003). Several studies showed that dairy LAB were capable of producing CLA *in situ* during fermentation of milk. However, this ability was interspecies dependant as well as some other factors such as culture concentration and fermentation time (Kim and Liu 2002; Coakley and others 2003).

51.6 HEALTH BENEFITS OF FERMENTED MILK

51.6.1 Alleviation of Lactose Intolerance

The decline of the intestinal β-galactosidase (β-gal; commonly know as lactase) activity is a biological characteristic of the maturing intestine in the majority of the world's population (Lee and Krasinski 1998). With the exception of the inhabitants of northern and central Europe and Caucasians in North America and Australia, over 70% of

adults worldwide are lactose malabsorbers (de Vrese and others 2001). Upon ingestion of lactose, it is hydrolyzed by lactase in the brush border membrane of the mucosa of the small intestine into constitutive monosaccharides, which are readily absorbed in the blood stream (Shah and Jelen 1991). However, the activity of the intestinal lactase in the lactose intolerant individuals is usually less than 10% of childhood levels (Buller and Grand 1990). This decline, termed hypolactasia, causes insufficient lactose digestion in the small intestine, characterized by an increase in blood glucose concentration or hydrogen concentration in breath upon digestion of 50 g lactose, conditions designated as lactose maldigestion (Scrimshaw and Murray 1988). Hypolactasia and lactose malabsorption accompanied with clinical symptoms, such as bloating, flatulence, nausea, abdominal pain, and diarrhea, are termed lactose intolerance. Symptoms are caused by undigested lactose in the large intestine, where lactose is fermented by intestinal microflora and osmotically increases the water flow into the lumen. The severity of the symptoms depends primarily on the size of the lactose load ingested (Savaiano and Levitt 1987). The development of the intolerance symptoms also depends on the rate of lactose transit to the large intestine, influenced by the osmotic and caloric load, and the ability of the colonic microflora to ferment lactose (Martini and Savaiano 1988). Lactose intolerance usually leads to self-imposed dietary restrictions of dairy products, which in turn may have deleterious nutritional consequences in later life, such as low calcium absorption resulting in osteoporosis (Pietschmann and others 1991).

Numerous studies have shown individuals with hypolactasia could tolerate fermented dairy products better than an equivalent quantity in milk (Martini and others 1987a,b; Rosado and others 1992). Various explanations have been suggested in order to clarify this phenomenon. At least three factors appear to be responsible for a better tolerance of lactose in fermented milk including (1) starter culture, (2) intracellular enzyme β-galactosidase or lactase expressed in these cultures, and most importantly, (3) oro-caecal transit time. The traditional cultures used in dairy fermentations utilize lactose as an energy source during growth thus at least partially reducing its content in fermented products. Furthermore, the bacterial lactase may resist luminal effectors avoiding denaturation and can be detected in the duodenum and terminal ileum after consumption of products containing live bacteria. The presence of this enzyme may lead to lactose hydrolysis and improved lactose tolerance (Martini and others 1991). On the other hand, other studies could not support this theory finding no difference in digestion and tolerance to lactose in several fermented dairy products with substantially different lactase activities (Vesa and others 1996). It was suggested that increased viscosity of fermented milk, in this case yogurt, slowed gastric emptying and consequently prolonged transit time through the gastrointestinal tract improving absorption and lactose tolerance (Shah and others 1992).

51.6.2 Antiosteoporotic and Antiobesity Effect

In addition to major macronutrients, fermented milk are excellent source of important minerals, namely calcium and phosphorus and several vitamins, especially those belonging to the B group. In general, dairy products provide for up to 75% of recommended dietary intake of calcium (Narva and others 2004b). Calcium plays a major role in bone formation and mineralization and daily requirements are influenced by age, pregnancy, and lactation. However, recent studies showed an acutely low calcium intake in pregnant women (Adolfsson and others 2004). Additionally, postmenopausal women

are at greater risk due to absent estrogen activity that otherwise governs bone recalcification (Heaney 2000). Due to low pH in fermented milk, calcium is readily solubilized and presented in its ionic form. Initially it was believed that this predominant factor influenced a good bioavailability of dairy calcium. However, the evidence has been rather contradictory. Some authors pointed out the role caseinophosphopeptides (CPP), small proteinaceous fractions liberated by proteolytic activity of either gastrointestinal or microbial proteases, in mineral binding and adsorption. Several *in vitro* studies have shown that these phosphorylated peptides were capable of binding and solubilizing calcium, thus preventing precipitation in the presence of phosphates at physiological pH (Holt and others 1996, 1998). However, *in vivo* studies have not been able to confirm these findings. Fermented milk indeed enhanced the calcium absorption, which was substantially higher in comparison to that of unfermented milk; however, the mechanisms still remained unclear with CPPs playing no apparent role (Narva and others 2003, 2004a,b).

In addition to prevention of osteoporosis, preliminary findings suggest that dairy products as a part of an energy-controlled diet may prevent obesity by enhancing the weight loss. The prevalence of obesity has increased dramatically over the years. The latest National Health and Nutrition Examination Survey, analysing data from 1999 to 2002, showed that one-third of all adults (at least 20 years of age) in the United States were obese with an additional one-third being considered overweight (Hedley and others 2004). As demonstrated by several epidemiological studies (Heaney and others 2002; Skinner and others 2003; Zemel and others 2004), after controlling for energy intake and physical activity, body fat was lower in people with the highest calcium intake. The risk for obesity decreased with an increase in calcium intake, reaching 85% at the RDI level. Studies also showed that fermented dairy products produced a superior effect on both fat loss and fat distribution in comparison to equivalent amounts of supplemental calcium. Various mechanisms have been proposed to explain this inverse relationship between dairy consumption and body mass index (BMI). Apparently, intracellular calcium plays a major role in modulation of human adipocyte metabolism. Higher levels of calcitrophic hormones in blood, resulting from a low-calcium intake stimulate the influx of calcium into human adipocytes. Consequently, this influx causes a metabolic switch from lipolysis to lipogenesis, thus fat is rather stored than burnt (Zemel 2003). On the other hand, concentrations of calcitrophic hormones may be maintained at low level by increasing calcium intake, reversing the calcium flow and therefore increasing lipolysis. Although dairy consumption may not be a sole answer to the prevalence of obesity, owing its multifactorial dependence, these findings indicate that dairy products may present an important part in the prevention of obesity accompanied with other dietary measures and physical activity.

51.6.3 Physiological Activity

Dairy starter cultures used in production of fermented milk have appreciable proteolytic activity enabling their rapid growth in milk. During fermentation, milk proteins, namely caseins, undergo a slight proteolytic degradation resulting in a number of potentially bioactive peptides (Table 51.3). Strains of *Lactobacillus helveticus* have been identified as highly proteolytic and peptidolytic towards caseins and their derivatives and, if used in fermentations, they can produce products with a

TABLE 51.3 Some Examples of the Identified Bioactive Peptides in Fermented Milk and Their Corresponding Physiological Activity (Gobbetti and others 2000; Hernandez-Ledesma and others 2005; Nakamura and others 1995; Robert and others 2004; Seppo and others 2003; Yamamoto and others 1999).

Sequence	Microbial Agent	Precursor	Bioactivity
Val-Pro-Pro, Lle-Pro-Pro	*Lb. helveticus* CM4 and *S. cerevisiae*	β-and κ-casein	Hypotensive
Val-Pro-Pro, Lle-Pro-Pro	*Lb. helveticus* LBK16H	β-and κ-casein	Hypotensive
Phe-Pro-Glu-Val-Phe-Glu-Lys	Commercial products and digestion	α_{s1}-casein	ACE inhibition
Lys-Val-Leu-Pro-Val-Pro-Glu	Commercial products and digestion	β-casein	Antioxidative
Lys-Thr-Thr-Met-Pro-Leu-Trp	Commercial products and digestion	α_{s1}-casein	Possible immunomodulation
Asn-Leu-His-Leu-Pro-Leu-Pro-Leu-Leu	*Lb. helveticus* NCC 2765	β-casein	ACE inhibition
Tyr-Pro-Phe-Pro-Glu-Pro-Ile-Pro-Asn	*Lb. helveticus* NCC 2765	β-casein	Opioid
Tyr-Pro	*Lb. helveticus* CPN4	caseins	ACE inhibition
Leu-Asn-Val-Pro-Gly-glu-Ile-Val-Glu	*Lb. delbrueckii* ssp. *bulgaricus* SS1	β-casein	ACE inhibition
Asn-Ile-Pro-Pro-Leu-Thr-Glu-Thr-Pro-Val	*Lc. lactis* ssp. *cremoris* FT4	β-casein	ACE inhibition

range of bioactivities such as opioid and hypotensive activity (Maeno and others 1996; Matar and others 1996).

Casein- and potentially whey proteins-derived bioactive peptides released through the proteolytic action of dairy starters may function as regulatory compounds or exorphins. These peptides with a morphin-like activity may act as opioid agonists such as α- and β-casomorphins, and lactorphins or opioid antagonists presented by casoxins. The opioid peptides are generally short containing between five and 10 amino acid residues. They have the ability to bind to opioid receptors on intestinal epithelial cells exhibiting a range of physiological functions such as modulation of social behavior, antidiarrheal action and stimulation of endocrine responses (Clare and Swaisgood 2000). Casomorphins appear to be resistant to digestion by gastrointestinal enzymes expressing an appreciable activity in the gut (Trompette and others 2003). This activity slows down the rate of the gastric emptying enhancing the uptake rate of amino acids and electrolytes by epithelial cells. Casomorphin activity is dependant on consensus amino acid sequences that require the presence of a Tyr residue at the N-terminal end and presence of another aromatic amino acid residue, Phe or Tyr, in the third or fourth position (Clare and Swaisgood 2000).

Another group of bioactive peptides, termed angiotensin I-converting enzyme (ACE) inhibitors, have been extensively studied due to their hypotensive role. Hypertension is a risk factor in the development of a number of cardiovascular diseases such as stroke and coronary infarction. The risk of acquiring these diseases is dramatically diminished by even slight reduction in diastolic pressure, which can be achieved by certain dietary modifications (Conlin and others 2000). In some cases, drug treatments may be employed producing a range of effects such as vasodilation and inhibition of angiotensin I-converting enzyme (ACE, EC 3.4.15.1) activity. Blood pressure is controlled by different interacting biochemical pathways but, in general, it is associated with the renin-angiotensin system. This system begins with the inactive precursor angiotensinogen,

a glycopeptide that is the only known precursor of angiotensin I as well as the only known substrate for renin (EC 3.4.23.15). Renin is responsible for liberation of angiotensin I from angiotensinogen (Inagami 1994). Angiotensin I, the decapeptide released from the N-terminal portion of angiotensinogen, is further cleaved by ACE, which removes the C-terminal dipeptide Hys-Leu, resulting in the formation of angiotensin II, a potent vasoconstrictor. At the same time, ACE removes the C-terminal dipeptide from bradykinin, a potent vasodilator, resulting in the formation of inactive peptide fragments (Inagami 1994).

Milk proteins contain a number of ACE inhibiting peptides encrypted within their primary structures. These peptides are liberated by proteolytic action of extracellular and intracellular enzymes of LAB proteolytic system. Recently several studies surveyed commercial fermented milk products on the European market in regard to ACE inhibitory activity (Hernandez-Ledesma and others 2004, 2005). The moderate inhibition was observed depending on the origin of milk and strains employed. Surprisingly, the ACE activity was not further suppressed by peptides released after additional treatment with pancreatic enzymes. On the contrary, Pihlanto-Leppälä and others (1998) reported that the *in vitro* release of ACE inhibitory peptides from casein or whey by yoghurt cultures required further incubation with pepsin and trypsin. Strains of *Lactobacillus helveticus* appeared to be superior in regard to production of ACE inhibitory peptides in comparison to other species tested (Leclerc and others 2002; Ashar and Chand 2003; Fuglsang and others 2003). Several commercial products containing highly proteolytic strains of *L. helveticus* were developed and products have been marketed as possessing hypotensive activity. One of them, Calpis® sour milk (Calpis Co. Ltd., Tokyo, Japan), is prepared by fermenting milk using mixed culture containing *L. helveticus* CM4 (CP790) and *Saccharomyces cerevisiae*. These organisms are responsible for a release of a range of potent bioactive peptides including two tripeptides, Val-Pro-Pro and Ile-Pro-Pro (Nakamura and others 1995). Another antihypertensive fermented milk is marketed by Valio Ltd. (Finland) under Evolus® brand. The brand covers a range of fruit flavored, sugar-free products produced by fermenting ability of the highly proteolytic *L. helveticus* LBK-16H. The strain produced similar amounts of hypotensive tripeptides as *L. helveticus* CM4, but it substantially delayed the development of hypertension in spontaneously hypertensive rats indicating additional contribution of other product constituents (Sipola and others 2002). Fermented milk produced with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus lactis* spp. *cremoris* were also shown to contain ACE inhibitory peptides (Gobbetti and others 2000; Ashar and Chand 2004). Only a limited number of human studies have been performed on the antihypertensive activity of commercially available fermented milk products. In 1996, Hata and others (1996) designing a double-blinded placebo controlled study, demonstrated that ingestion of 95 mL per day of fermented sour milk (Calpis®) significantly reduced diastolyc and systolic blood pressure in mildly hypertensive human volunteers. Similar observations were made in other independent studies on the ingestion of fermented milk (150 mL per day) containing similar quantities of hypotensive tripeptides, Val-Pro-Pro and Ile-Pro-Pro, although the overall effect was modest (Seppo and others 2002, 2003; Tuomilehto and others 2004).

51.7 FUTURE TRENDS

It has become evident that fermented milk possesses a range of physiological activities beyond those provided by probiotics, prebiotics and symbiotics. The research is still

ongoing and it is expected to see more biogenic substances identified covering a broader range of activities than encountered today. However, this trend will ensue important questions that should resolve several scientific and regulatory as well as technological issues. The current technological methods may not be adequate to efficiently identify, extract, and concentrate bioactive components calling for innovative and novel techniques. The effect of processing parameters and/or their optimization for an improved functionality of bioactives is largely unknown. The safety of derived peptides and other compounds formed during fermentation needs to be assessed as well. More *in vitro* and *in vivo* animal and human studies are required to fully elucidate the effects of these compounds. The development of new molecular methods is needed to elaborate the mechanism(s) underlying the activity of a wide range of bioactives. Evidently, fermented milk proves to be an important component of diet not only providing for major nutrients but also minimizing risk associated with a range of chronic conditions and diseases.

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52

Application of Functional Foods and Nutraceuticals in Allergic Disorders: Principles and Potential

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52.1 Introduction 118
52.2 Classification and Prevalence of Allergic Disorders 118
52.3 Allergic Disorders: Mechanisms 119
52.4 Functional Foods and Allergic Diseases 120
52.4.1 Fish Oil and its Components 120
52.4.2 Probiotics and Allergy 122
52.4.3 Breast Milk: A Natural Functional Food Against Allergic Disorders? 124
52.5 Nutraceuticals and Allergy 125
52.5.1 Minerals 126
52.5.2 Vitamins 127
52.6 Summary, Challenges, and Future Directions 127
References 128

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

52.1 INTRODUCTION

Allergic disorders include a range of health problems including food and drug allergies, allergic rhinitis, allergic conjunctivitis, allergic dermatitis, allergic asthma, and systemic anaphylaxis. Allergies are also referred to as "atopic disorders" (atopy in Greek meaning "unusual"). This historical nomenclature of atopy as unusual conditions contrasts with the present global epidemic of allergies and asthma (ISAAC 1998; Bousquet and others 2004; Johansson and others 2004; Masoli and Beasley 2004).

Based on the time it takes for the clinical reaction to occur following exposure to the inciting agent, Coombs and Gell (1975) originally classified hypersensitivity diseases into four groups: Types I (immediate hypersensitivity), Type-II (cytotoxic reactions), Type-III (immune complex reactions), and Type- IV (delayed or cell mediated reactions). Accordingly, most allergies are generally regarded as Type-I diseases with some drug reactions being Type-II. Notably, although called "contact allergy", reactions to poison ivy or nickel are delayed reactions that belong to Type-IV category. Although allergies in general are regarded as Type-I hypersensitivities, recent advances reveal that atopic eczema, allergic asthma, and food allergies may involve both Type-I and Type-IV components (Johansson and others 2004; Sampson 2004).

Functional foods and nutraceuticals is a rapidly evolving biomedical area focused on finding diet related solutions to a variety of human diseases. Although there is not one universally acceptable definition of these terminologies, in this chapter we would like to use them to indicate the health effects of some foods and nutrients that are beyond their basic nutritive value. Most of the literature on functional foods has used these terms in a positive context of their impact on health. However, inclusion of foods with negative impacts in addition to foods with positive impact on health, in the definition of functional foods would be more holistic. For example, allergenic foods that cause food allergies might be argued as functional foods with negative impact on health.

In this chapter, the current literature on the potential of most commonly studied functional foods and nutraceuticals for use in allergic disorders has been reviewed.

52.2 CLASSIFICATION AND PREVALENCE OF ALLERGIC DISORDERS

In order to reduce the confusion on the actual meaning of various terminologies used in the allergy field, a revised nomenclature for allergy for global use has been recently proposed by the Nomenclature Review Committee of the World Allergy Organization (Johansson and others 2004). A summary of the proposed definitions for various allergy related terminologies including hypersensitivity, allergy, atopy, allergen, and anaphylaxis has been provided in Table 52.1.

The World Allergy Organization (WAO) Nomenclature Review Committee has classified allergic diseases into nine different clinical conditions: asthma, rhinitis, conjunctivitis, dermatitis, urticaria, food hypersensitivity, drug hypersensitivity, insect sting or bite hypersensitivity, and anaphylaxis (Johansson and others 2004).

Although the prevalence of allergic disorders is increasing globally, elevated prevalence has been reported primarily among the industrialized countries (ISAAC 1998). Accordingly, the overall prevalence of allergic rhinitis, asthma and atopic dermatitis in

TABLE 52.1 Newly Evolved Definitions of Allergy Related Terminologies.

Terminology	Definition and Comments
Hypersensitivity	Objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons
Allergy	A hypersensitivity reaction initiated by specific immunologic mechanisms
Atopy	A personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins This definition establishes and reconfirms a central role for IgE antibodies as measured in the serum or by a positive skin prick test as key criteria for atopy
Allergen	Antigen causing allergic disease
Anaphylaxis	A severe, life-threatening generalized or systemic hypersensitivity reaction

Source: Johansson and others (2003).

developed countries (United States, United kingdom, Australia, and Canada) ranges from $\sim$10% up to 40%.

Food allergies are also becoming more common, especially in the developed countries. Recent data suggests a 100% rise in the prevalence of peanut allergy during the past decade in the United Kingdom and United States (Grundy and others 2002; Sicherer and others 2003; Sampson 2004). The overall prevalence of food allergies in general has been reported to be at 6% among children and at 3.7% among adults in the United States (Sampson 2004).

Scientific reasons for the dangerous trends of global allergy epidemic are unclear at present with a number of environmental factors being suspected as discussed later in this chapter.

52.3 ALLERGIC DISORDERS: MECHANISMS

Allergies in general are regarded as complex genetic diseases that are known to run in families (Hoffjan and Ober 2002). Several predisposing genes together with a variety of environmental factors appear to interact in many complex ways with the final clinical outcome as allergy or clinical tolerance to allergens.

Earlier observations that hayfever was rare among children born in larger sized family led to the "hygiene hypothesis" that has triggered an enormous amount of basic and applied research in the field with a focus on the role of the environment in allergy (Strachan 1989). Although it is controversial at present, this hypothesis appears to offer a unified framework to explain a number of environmental contributors to allergy development.

A number of environmental factors are suspected to play a role in the rising prevalence of allergies including: (1) environmental pollutants such as diesel exhaust particles, polychlorinated biphenyls, and tobacco smoke; (2) increased use of antibiotics especially during infancy/childhood; (3) reduced exposure to childhood infections; (4) growing up in nonfarming or urbanized environment during early childhood; and (5) alterations in the bowel microbiota due to reduced breast feeding, or use of antibiotics (Strachan 1989; Shirakawa and others 1997; Crane 2002; Diaz-Sanchez and others 2003; Kabesch and Lauener 2004; Kaiser 2004).

Allergic diseases are mediated by the immune system that normally plays a role in host defense. In this case, the immune system reacts to innocuous environmental agents (e.g., food, pollen, animal dander, insect feces/saliva, etc.) in such a way that the outcome is detrimental to the host rather than protection.

Extensive basic research in this area has identified key molecular and cellular factors in the pathogenesis of allergies. These include: IgE class of antibodies, Type-2 (or T-helper-2) cytokines such as IL-4, IL-5, IL-13, Type-2 associated chemokines such as eotaxin, TARC, MCP-4; B cells producing IgE antibodies, T-helper-2 cells, eosinophils, basophils, and mast cells (Gangur and Oppenheim 2000; Campbell and others 2002; Gangur and others 2003; Romagnani 2004).

52.4 FUNCTIONAL FOODS AND ALLERGIC DISEASES

In this section literature on fish oil, probiotics and breast milk as it relates to allergies has been reviewed.

52.4.1 Fish Oil and its Components

Arachidonic acid pathway of fatty acid metabolism plays a key role in the generation of mediators of inflammation such as prostaglandins, thrombaxanes, and leukotrienes. It is generally thought that the metabolic end products of the omega-6 pathway promote inflammation as opposed to the antiinflammatory nature of the end products of the omega-3 pathway of arachidonic acid metabolism. Consequently, in principle, promoting the omega-3 pathway may competitively inhibit the potential of omega-6 pathway mediated promotion of inflammation. One way of accomplishing this goal would be to use omega-3 (or n-3) fatty acids derived from dietary alpha-linolenic acid (18 : 3n-3) or from marine sources such as eicosapentaenoic acid (20 : 5n-3) (EPA) and docosahexaenoic acid (22 : 6n-3) (DHA). The latter fatty acids have been regarded as the active health promoting (or antiinflammatory) components of certain fish oils in particular.

There has been growing interest in the potential of fish oil use for treatment or prevention of allergic and other chronic inflammatory disorders. However, the outcomes from various studies have been controversial (Table 52.2).

In a prospective study examining the capacity of fish oil to prevent development of allergeies, the relation between introduction of fish in the diet during the first year of life, and risk of asthma and allergic rhinitis, was assessed in cohort study of 2531 Norwegian children. The introduction of fish in the diet during the first year of life resulted in the adjusted OR for allergic rhinitis of 0.45 (95% confidence interval [CI] = 0.28, 0.74) and for asthma 0.84 (95% CI = 0.57, 1.22) (Nafstad and others 2003).

A randomized control study examined the impact of dietary supplementation with fish oil for 10 months in 29 children with preexisting bronchial asthma (Nagakura and others 2000). To avoid exposure to pollen, the study was conducted in a long-term treatment hospital set-up. They used fish oil capsules containing EPA (84 mg) and DHA (36 mg), or control capsules containing 300 mg olive oil per day. They concluded that dietary supplementation with fish oil rich in EPA and DHA is beneficial for children with bronchial asthma based on decreased symptom scores and responsiveness to acetylcholine. However, mechanism of action was not studied.

TABLE 52.2 Fish Oil and Allergy: Summary of Major Studies.

Type of Disease	Type of Intervention	Subjects	Conclusion	Reference
Atopy	Level of n-3 PUFA in breast milk and risk of atopy in breast fed infants	Infants (up to 2 years)	Higher n-3 PUFA, DHA and DPA in colostrum associated with *increased* atopy	Stoney and others (2004)
	Plasma n-3 PUFA levels vs. atopy/asthma risk	Adults (~35 years)	Plasma n-3 PUFA are not associated with reduced asthma or atopy	Woods and others (2004)
	Maternal dietary supplements with n-3 PUFA during pregnancy vs. neonatal immune response to allergens	Infants (1 year)	3× less likely to be atopic to egg	Dunstan and others (2003)
	Dietary n-3 FA ± avoidance of HDM of pregnant mothers	Infants (up to 18 months)	No impact on readouts	Mihrshahi and others (2003)
Atopic Dermatitis	Maternal dietary supplements with n-3 PUFA during pregnancy vs. neonatal immune response to allergens	Infants (1 year)	No difference in frequency but less severe disease at 1 year age	Dunstan and others (2003)
	N-3 vs. n-6 PUFA lipid infusion iv	Adults (~23 years)	N-3 improved severity of AD	Mayser and others (2002)
Asthma	Plasma n-3 PUFA levels vs. atopy/asthma risk	Adults (~35 years)	Plasma n-3 PUFA are not associated with reduced BHR	Woods and others (2004)
	Fish oil consumption during first year of life	Children <1 year	Reduced risk of asthma in childhood	Nafstad and others (2003)
	Dietary n-3 FA ± avoidance of HDM by pregnant mothers	Infants (up to 18 months)	9.8% reduction	Mihrshahi and others (2003)
	Cochrane Database Review of Trials from 1986 to 2001	Children & adults	Little evidence in controlling asthma	Woods and others (2002)
	Dietary Supplementation with fish oil (EPA & DHA)	Children	Symptom scores decreased	Nagakura and others (2000)
	Asthma vs. dietary fish oil	Adults	Does not prevent asthma during pollen season	Thien and others (1993)
Hayfever	Fish oil consumption during first year of life	Children <1 year	Reduced risk of allergic rhinitis in childhood	Nafstad and others (2003)
	Dietary supplementation with fish oil in pollen allergic subjects	Adults	Does not prevent symptoms during pollen season	Thien and others (1993)

BHR, bronchial hyperresponsiveness; HDM, house dust mite; PUFA, polyunsaturated fatty acid; EIB, exercise induced bronchoconstriction; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AD, atopic dermatitis.

Another study tested the hypothesis that the incidence of asthma and allergy in high-risk children would be reduced by avoidance of HDM allergens, supplementation with omega-3 fatty acids, or the combination of these strategies (Mihrshahi and others 2003). They published the results of an interim analysis reporting outcomes assessed at 18 months. This study involved a total of 616 pregnant women who were randomized to an HDM avoidance intervention (accomplished by the use of impermeable mattress covers and an acaricide) or control and the use of an oil supplement, margarines, and cooking oils containing high levels of omega-3 fatty acids or control. Based on findings of 7.8% absolute reduction in the prevalence of wheeze ($p = 0.04$), they concluded that increasing dietary omega-3 fatty acids might have, beneficial effect. Notably, they found no major effects on serum IgE. Data from the follow-up study at five years is expected.

In contrast to above results, a community based cross-sectional study examining whether dietary n-3 PUFA intake differed between young adults with and without asthma concluded that neither plasma n-3 nor n-6 : n-3 ratio were consistently associated with asthma (as measured by BHR). (Woods and others 2004).

As opposed to numerous studies on asthma, relatively less has been studied in allergic rhinitis and atopic dermatitis or food allergies. One study reported that maternal dietary supplements with n-3 PUFA during pregnancy reduces the severity (but not frequency) of atopic dermatitis among infants born and that the infants are three times less likely to be atopic to egg (Dunstan and others 2003). Others reported that IV n-3-fatty acid administration is effective in acutely improving the severity of atopic dermatitis, paralleled by changes in plasma and membrane fatty acid composition and lipid mediator synthesis (Mayser and others 2002). They recommended further evaluation of long-term effects of this approach.

One study on allergic rhinitis (or hayfever) indicates that fish oil reduced the risk of disease among children (<1 year age) (Nafstad and others 2003). However, another study in adults during pollen season found no preventive effect of dietary fish oil (Thien and others 1993).

With the exception of one, studies examining atopy (i.e., allergen skin prick test positivity or serum IgE) as a readout conclude that n-3 PUFA has either had no impact on atopy (Mihrshahi and others 2003; Woods and others 2004) or higher n-3 PUFA (DHA and DPA) may increase the risk of atopy (Stoney and others 2004).

52.4.2 Probiotics and Allergy

Originally the term "probiotics" was coined to describe factors produced by protozoa that promote the growth of other protozoa (Lilly and Stillwell 1965). Later it was refined to refer to "a live feed supplement which beneficially affects the host animal by improving its microbial balance" (Fuller 1989).

Others have recently proposed a series of desirable criteria for probiotics to be useful in promoting health (Heyman and Menard 2002; Laiho and others 2002). These include: (1) resistance to acid and bile; (2) human origin; (3) ability to adhere to the intestinal epithelial cells; (4) ability to colonize and persist for long periods of time in the gut; (5) to produce antimicrobial substances; (6) to modulate immune responses; (7) desirable technological characteristics such as resist technological processes, good shelf life, etc.; (8) documented health benefits; and (9) safety. Notably, not every one in the field accepts whether all these criteria should constitute the definition of probiotics.

TABLE 52.3 Common Probiotic Bacteria for Potential Use in Functional Foods.

Genus	Species[a]
Bifidobacterium	*B. bifidum*
	B. longum
	B. adolescentis
	B. breve
	B. infantis
	B. lactis
Lactobacillus	*L.acidophilus*
	L. rhamnosus
	L. rhamnosus GG
	L. gasseri
	L. reuteri
	L. bulgaricus
	L. plantarum
	L. johnsonii
	L. lactis
	L. paracasei
	L. casei
	L. salivarius

[a]Boxed probiotic species have been tested in human allergic diseases.

Most frequently used probiotic microorganisms, typically in fermented dairy products, belong to the genuses *Lactobacillus*, *Bifidobacterium*, and *Steptococcus.* Common examples of species of probiotics are summarized in Table 52.3.

Potential application of probiotics in allergic diseases stems from the following two fundamental principles: (1) Population based studies (primarily epidemiological studies) suggest that increased exposure to bacteria early in life may have protective effects against allergies although the true nature of these bacterial species is unclear at present (Crane 2002; Prescott 2003; Kabesch and Lauener 2004). Probiotics are one set of candidate bacterial species implicated. (2) Certain probiotic bacteria have been shown to affect the immune response of the host in animal models and *in vitro* studies. For instance, some may modulate immune cell functions (Vaarala 2003) (Pestka and others 2001; Tejada-Simon and others 1999a,b). Since allergies are immune mediated diseases, probiotics offer promise in this context (Romagnani 2004).

These principles are supported by the following recent evidence: (1) Some clinical studies have shown that use of probiotics may help reduce the severity and frequency of atopic eczema in infants (Table 52.4) (Majamaa and Isolauri 1997; Isolauri and others 2000; Kalliomaki and others 2001, 2003); (2) use of antibiotics (presumably with consequent reduction or elimination of gut microflora) in infants is associated with increased allergies (Crane 2002; Wickens and others 1999); (3) some studies suggest that gut microflora of atopic vs. nonatopic subjects may differ in composition with the levels of *Clostridia* increased and the *Bifidobacterium* reduced among atopics (Kalliomaki and others 2001; Kirjavainen and others 2001; Ouwehand and others 2001; Laiho and others 2002).

Not all studies on the use of probiotics in allergy have revealed promise in treatment or prevention. Thus, one study found no significant changes in asthma during treatment with *Lactobacillus acidophilus* (Wheeler and others 1997). Another study found no

TABLE 52.4 Probiotics: Evidence for Potential Application in Atopic Eczema.

Type of Intervention	Outcome	Reference
Cow's milk formula fortified with *Lactobacillus rhamnosus* (strain GG)	Significant improvement in the clinical score of atopic eczema	Majamaa and Isolauri (1997)
Whey formula with *Bifidobacterium lactis* (Bb-12) or with *L. rhamnosus* (strain GG)	Significant improvement in the severity of atopic eczema	Isolauri and others (2000)
L. rhamnosus (strain GG) to infants or to mothers who breastfed their infants	Significant reduction in the incidence of atopic eczema during infancy (first two years of life)	Kalliomaki and others (2001)
L. rhamnosus (strain GG) to infants or to mothers who breastfed their infants	Preventive effect of the probiotic on atopic eczema extends beyond infancy (as assessed at four years of age)	Kalliomaki and others (2003)

major differences in symptomatic scores in allergic rhinitis/asthma when treated with *Lactobacillus rhamnosus* (Helin and others 2002).

While the reasons as to why probiotics were beneficial in some studies but not in others are not clear at present, there are a number of differences to consider: (1) age group of subjects (children vs. adults/adolescents); (2) species and strains of probiotic used (*Lactobacillus GG* vs. *L acidophilus*); and (3) the type of allergic disease readout tested (atopic eczema vs. allergic rhinitis/asthma). Clearly more studies are needed to replicate the above findings while addressing these potential variables.

52.4.3 Breast Milk: A Natural Functional Food Against Allergic Disorders?

Breast milk is the first wholesome food that the baby is expected to consume in a natural setting. Although breast milk is considered to be a food in general, available evidence in the literature would argue that it is a natural 'functional food' designed for the overall health and development of the baby. This is because, breast milk is enriched in not only food related components and nutrients but also a variety of immunoregulatory molecules as well as immune cells (Table 52.5) (Skansen-Saphir and others 1993; Kalliomaki and others 1999; Bottcher and others 2000, 2002; Hawkes and others 2002; Takahata and others 2003).

TABLE 52.5 Allergy Relevant Immunomodulators Present in Human Breast Milk.

Immunomodulator Group	Examples
Cytokines	IL-4, IL-5, IL-10, IL-13, IFN-γ, TGF-β1/β2,TNF-α
Chemokines[a]	CXCL8 (IL-8), CCL5 (RANTES), CCL11 (Eotaxin), CXCL10 (IP-10)
Lipid mediators	PGE2, LT
Antibodies	IgA1, IgA2

IL, interleukin; IFN, interferon; TGF, transforming growth factor; TNF, tumor necrosis factor; PG, prostaglandin; LT, leukotriene; IP, interferon inducible protein; RANTES, regulated upon activation naturally T cell expressed and secreted; Ig, immunoglobulin.

[a]Old names are indicated in parentheses.

The question of whether breast milk is protective against allergic disorders in particular has been addressed for more than two decades with equivocal evidence. There are several hypotheses related to breast milk and allergy that involve both protection as well as sensitization. One the major hypotheses in this area is that elevated levels of IgA antibodies bind to potential allergens in the infant's gut and facilitate their elimination, thereby preventing sensitization. This is supported by reports that low colostral IgA is associated with cow's milk allergy (Bottcher and others 2002; Savilahti and others 1991). Additional hypotheses implicate a role for immunomodulating molecules in breast milk such as cytokines in allergy (Skansen-Saphir and others 1993; Kalliomaki and others 1999; Bottcher and others 2000, 2002; Hawkes and others 2002; Takahata and others 2003) (Table 52.5).

There is evidence that exclusive breast feeding for four to six months is associated with reduced occurrence of eczema and asthma (Oddy and others 1999; Romieu and others 2000; Chandra 2002; Fiocchi and others 2003), although one study reports lack of such evidence (Rust and others 2001). Notably, this protection appears to be impaired by addition of cow's milk or soy formula to the diet (Chandra 2002a,b).

Food allergens such as peanut allergens can be passed on from the mother's circulation to the breast milk (Vadas and others 2001). Therefore, there is growing concern that breast milk may contain potentially hazardous sensitizing allergens that may pose a risk for the development of allergies among breast fed infants. Consequently, it may be prudent for pregnant/nursing mothers to avoid consumption of well established allergenic foods such as egg, peanut, tree-nuts, fish, and soy (Chandra 2002a,b; Fiocchi and others 2003).

There appears to be a general agreement that the late introduction of solid foods beyond six months after birth may delay/prevent development of allergies (Chandra 2002a,b; Fiocchi and others 2003). The mechanism of how this dietary method may prevent/delay allergies is unclear, but may be related to the immature gut physiology/immunology of the infants.

There has been enormous interest in developing hypoallergenic infant formulae. This approach is based on the principle that breaking-up of larger protein molecules into smaller pieces might eliminate the critical "epitope" structure required for immune recognition and allergy development. Accordingly, there is evidence that elemental or extensively hydrolyzed formulae may be of tremendous value in the management of food allergy (Agostoni and Haschke 2003).

52.5 NUTRACEUTICALS AND ALLERGY

The concept that certain nutrients may impact allergic disorders because of their antioxidant and/or immunomodulating properties continues to be explored. This is based on two underlying principles: (1) allergic disorders such as asthma and atopic dermatitis (AD) are associated with increased oxidative stress in the host (Kelly and others 1999; Antille and others 2002); and (2) allergic disorders result from overactive immune responses of particular types (e.g., T helper 2 dominated) (Gangur and Oppenheim 2000; Campbell and others 2002; Gangur and others 2003; Romagnani 2004).

These ideas have translated into a variety of investigations focused on three basic approaches: (1) to evaluate whether there is a fundamental *in vivo* deficiency of certain nutrients in asthma and AD; (2) to test the impact of dietary supplementation of such

nutrients on the disease; and (3) basic studies to evaluate the impact of selected nutrients on oxidative stress and immune functions using cell line and animal models. In this section we will restrict our discussions primarily to findings from human clinical studies.

52.5.1 Minerals

Common minerals examined in context of allergies and asthma includes: zinc, magnesium, and selenium. Certain minerals form part of the natural antioxidant defense mechanism of the body. Zinc, copper, and manganese are components of super oxide dismutase; and selenium is a component of glutathione peroxidase. Consequently much interest has been to evaluate the *in vivo* status of these minerals in allergy.

It is well known that the deficiency of zinc can result in dermatitis. Consequently, the role of zinc in atopic dermatitis has been studied. Zinc is often used in skin ointments and creams with clinical benefit. Although studies reveal reduced levels of zinc in the serum of children with atopic eczema (David and others 1984), others report no significant impact of oral zinc supplementation on atopic dermatitis (Ewing and others 1991).

Studies using a magnesium deficient mouse model revealed a role for this micronutrient on the host inflammatory and immune responses as well as apoptosis (Tam and others 2003). Fantidis and others (1995) have proposed that magnesium inhibits contraction of bronchial smooth muscle and histamine release – two key pathogenic functions involved in asthma (Fantidis and others 1995). Another study has shown that intravenous magnesium supplementation could alleviate symptoms in acute as well as chronic asthma (Monteleone and Sherman 1997). There is a study reporting that intracellular Mg decreases in leukocytes on the first day of asthma attack, but after five days of anti-asthma therapy (with salbutamol), these levels return to normal values (Mircetic and others 2001).

Inhaled magnesium has been shown to inhibit methacholine-induced bronchoconstriction (Rolla and others 1987). A large scale study of adults ($n = 2633$) in the United Kingdom found that increased intake of Mg (100 mg/day) resulted in decreased symptoms of asthma as reported by the patients (Britton and others 1994). In a small study, magnesium picolinate oral administration three times a day for one month to subjects with hayfever appeared to be beneficial, although a larger study is warranted to further evaluate this (Cipolla and others 1990).

The above results are in contrast to a recent negative outcome from a randomized, placebo-controlled, double-blind parallel group trial of 16 weeks supplementation with 450 mg/day magnesium chelate or matched placebo in 300 asthma patients aged 18–60 years with physician-diagnosed asthma (Fogarty and others 2003).

Examination of the blood of asthma patients has revealed reduced levels of selenium as opposed to controls (Stone and others 1989; Kadrabova and others 1996; Misso and others 1996). One study examining 100 mg of sodium selenite daily for 14 weeks revealed subjective improvement in asthma (Hasselmark and others 1993). However, another study showed no effect on the clinical severity of atopic dermatitis (Fairris and others 1989).

Thus, there is controversial evidence on the role of micronutritents as a therapeutic agent for allergies and asthma. Although there is some potential based on some studies with positive findings, more studies are needed to make a definitive conclusion on the utility of micronutrients in the treatment of allergies and asthma.

52.5.2 Vitamins

Vitamin C, vitamin E and alpha-carotene are implicated in allergies and asthma primarily because of their antioxidant activities. Many have examined the serum profile of antioxidants in patients with asthma or allergies. Thus, in one study asthma diagnosis was associated with lower levels of serum vitamin C, alpha-carotene, beta-carotene, and beta-cryptoxanthin (Harik-Khan and others 2004).

Studies examining the impact of vitamin C on exercise induced asthma, skin, and nasal allergy have all failed to reveal a significant impact on the disease (Britton and others 1995; Fortner and others 1982). Kaur and others (2001) performed a systematic review of literature on the role of vitamin C in asthma and concluded that evidence from randomized-controlled trials is insufficient to recommend a specific role for vitamin C in the treatment of asthma (Kaur and others 2001).

There has been more promising data on the impact of vitamin E on allergies and asthma. A large study (77,866 women) examined the incidence of asthma and intake of vitamin E via diet vs. supplements over a 10-year period. Women in the highest quintile of vitamin E intake from diet, but not from supplements, had a risk of 0.53 (95% confidence interval [CI] = 0.33–0.86) compared with women in the lowest quintile. This suggests of a modest protective effect of vitamin E from the diet (Troisi and others 1995).

More recent studies have revealed the following: (1) higher concentrations of vitamin E intake were inversely associated with serum IgE and lower frequency of atopy (Fogarty and others 2000); (2) Nagel and others (2003) reported that increasing intake of vitamin E had a protective effect on hay fever (OR: 0.38; n = 334 test vs. 1336 control subjects) especially among women and ex/current smokers (Nagel and others 2003); (3) another study concluded that the correlations between vitamin E intake (400 IU/day), IgE levels, and clinical symptoms indicates that vitamin E could be an excellent therapeutic tool for atopic dermatitis (Tsoureli-Nikita and others 2002); and (4) another study suggested that vitamin E intake may reduce adverse effects of air-pollutants such as ozone in subjects with asthma (Trenga and others 2001; Romieu and others 2002).

Thus, studies have revealed potential for the use of zinc, magnesium, selenium, and vitamin E in specific types of allergy phenotypes. It is also possible that other phytochemicals with antioxidant activities might be beneficial in these diseases. In this context, intake of 30 mg/day of lycopene for one week had significant protective effects against exercise induced asthma (Neuman and others 2000). Thus, there is wide scope for more research on the topic and more exciting discoveries are likely to be on the horizon.

52.6 SUMMARY, CHALLENGES, AND FUTURE DIRECTIONS

The current status of the field supports the concept that there is strong potential for certain probiotics, fish oil (specifically its constituents EPA and DHA), some minerals (zinc, magnesium), and vitamin E, to serve as components of novel anti-allergy functional foods with potential preventive and/or therapeutic applications (Fig. 52.1).

There are a number of challenges that this exciting research area faces including but not limited to the following: (1) Allergic disorders are a complex group of diseases; therefore, it is possible that specific nutraceutical/functional foods will not necessarily be applicable to all types of allergies. Consequently, studies need to be carefully designed, evaluated and interpreted so that the specific combination of nutraceuticals/functional foods could be used for appropriate types of allergies. For example, *Lactobacillus GG* may be useful in

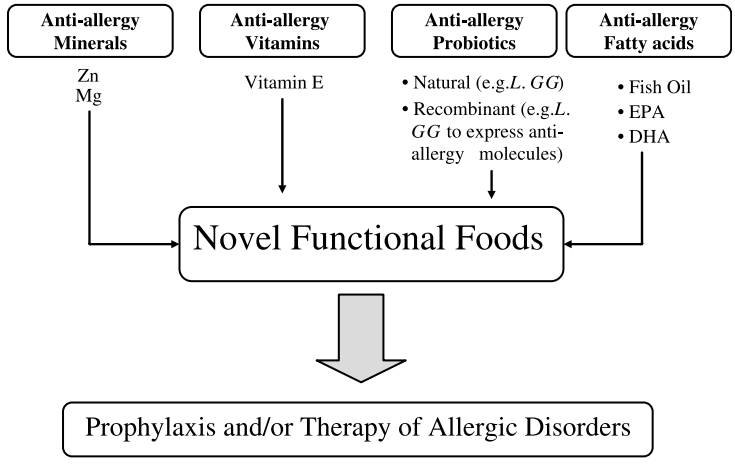

Figure 52.1 *An overview of the approach to develop novel functional foods for potential prophylaxis and/or therapy of allergic disorders.*

atopic dermatitis but unlikely to be useful in allergic rhinitis (Kalliomaki and others 2001, 2003). Similarly recent findings report that breast feeding was protective against some asthma phenotypes but not hay fever, atopic eczema or IgE (Karmaus and others 2003). (2) There is enormous amount of work ahead to establish variables including effective vs. toxic dose-ranges (for vitamins, minerals, EPA, DHA), effective combination of different nutraceuticals/functional foods, matrix effects, route of administration, impact of food processing, duration of intervention, and age and gender dependent differences. (3) It is unlikely that any novel preventive and therapeutic approach would be widely accepted unless underlying mechanisms of action (especially impact on relevant immune functions) is clearly demonstrable. Clearly, *in vitro*, animal model and human immune function studies should prove useful to address this issue.

Thus, despite several challenges, the area of functional foods and nutraceuticals vs. allergy is poised to make significant changes in the way we perceive food, diet, and human health in the 21st century.

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53

Functional Foods, Herbs, and Aging

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53.1 Introduction 136
53.2 Specific Antiaging Mechanisms 136
53.2.1 Antioxidant Activities 136
53.2.2 Aging Impairs Cardiac and Vascular Functions 137
53.2.3 Metal-Chelating Activities 138
53.3 Nonspecific Antiaging Mechanisms 138
53.3.1 Preventing Cataract and Eye Macular Degeneration (MD) 138
53.3.2 Cardiovascular Protection 140
53.3.3 Neuroprotection by Herbs and Functional Foods 141
53.3.4 Anticancer Activities 141
53.4 Functional Foods and Herbs: Towards a Healthy Diet 141
53.4.1 Fish, Fish Oil, and Olive Oil 141
53.4.2 Soy Foods: A Look to the Orient 142
53.4.3 Cocoa and Chocolate 142
53.4.4 Calcium and Vitamin A: New Functions for "Old" Nutrients? 142
53.4.5 Curcumin (Diferuloylmethane) 143
53.4.6 Propolis: An Aid from Bees? 143
53.4.7 Intake of Fruit and Vegetable Fibers Protects Against Gastrointestinal Cancers 143
53.4.8 Eat Tomato, Broccolis, and Garlic . . . Daily! 143

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

53.4.9 Grapes and Wine 144
53.4.10 Neuroprotective Effects of Teas (*Camellia sinensis*) and Coffees (*Coffea arabica*) 144
53.5 Conclusion 145
References 145

53.1 INTRODUCTION

In 1991, the Japanese Ministry of Health and Welfare first used the term "Foods for Specified Health Use" (FOSHU), a concept that was further coined by functional food in a Nature's article (Arai and others 2002). Functional food is considered any food able to enhance body functions improving health and decreasing disease risk. Detailed description regarding the concepts was done in Chapter 1.

Cognitive decline, cardiovascular disease, oxidative stress, inflammation, hypertension, and studies with health centenarians comprise important topics on aging research and practice (Pahor and Applegate 1997; Morley 2004).

A successful life span depends on many biological strategies to foster cell survival and preserve against many pathological stimuli. For useful purposes, biochemical and physiological protective pathways provided by functional foods can be separated in specific and nonspecific antiaging mechanisms.

53.2 SPECIFIC ANTIAGING MECHANISMS

As the study of functional foods is very recent, many controversial aspects could arise and the matter is far from a consensus. However, specific antiaging mechanisms of functional foods were recently suggested (Ferrari 2004) and are summarized in Table 53.1.

53.2.1 Antioxidant Activities

Antioxidant defense is essential to provide resistance against increased levels of oxidative stress biomarkers during the aging process (Ames and others 1993; Beckman and Ames 1997; Berllett and Stadtman 1997). Reinforcing the antioxidant-life span relationship, higher levels of vitamin A and E were found in human healthy centenarians (Mecocci and others 2000). Although some studies regarding antioxidant supplementation have reported positive effects on organisms' longevity, results are conflictive. Rather than increasing life span, antioxidants' benefits are related to the control of free radicals and mitochondrial stabilization that negatively influence healthy aging (Driver 2003; Le Bourg 2003).

Since oxidative stress induces both apoptosis (or programmed cell death) and necrosis (accidental cell death) leading to neuronal, cardiovascular, hepatic, ocular, renal, and other massive cell and tissue losses (Ferrari 2000a,b), antioxidant protection seems to be an essential protective antiaging strategy. Aging compromises mitochondrial membrane stability increasing cytochrome c releasing and reactive oxygen species (ROS) overload (Yan and Sohal 1998; Ferrari 2000a,b; Kokoszka and others 2001). Ishii and others

TABLE 53.1 Antiaging Specific Mechanisms Performed by Functional Foods.

Cellular and Molecular Activities	Cell Targets	Biomarkers
Antioxidation	Decreasing lipid peroxidation of cell membranes	Phospholipid bilayer; Mitochondrial external membrane (cytochrome c) and Bcl-2 protein
	Estimulation of the intracellular antioxidant defense systems	Antioxidant-response element (ARE), SOD, catalase, GSH, GPX, hemeoxygenase, GST, etc.
	Genome protection against reactive species (from oxygen, nitrogen, chlorine)	Oxidized DNA biomarkers
Antiapoptotic	Avoid membrane disruption and triggering of cell death machinery	Membrane death domains; Cytochrome c;
		Expression of Bcl-2 and other survival factors
Metal chelation	Chelation of iron, copper, and zinc excess to avoid mitochondrial damage and DNA oxidation	Cytochrome c; DNA oxidation products
Proapoptotic	Disruption of antioxidant defense and activation of apoptosis in oncogenic cells	Blockade of ARE; Cytochrome c releasing; Caspase activation
Mitochondrial stabilization	Protection of mitochondrial structures against damage	Apoptotic markers;
	Enhancement of mitochondrial function	ATP synthase activity, etc.

(2004) demonstrated that coenzyme Q_{10} and vitamin E, potent antioxidants, and mitochondrial stabilizers, had prolonged life span of *Caenorhabiditis elegans* worm by decreasing oxidative stress-induced apoptosis. The same protection is performed by melatonin (Reiter and Tan 2002).

Besides displaying an efficient control of cell death, antioxidants can provide two other protective mechanisms: (1) triggering expression of antioxidant genes (expression of superoxide dismutase enzyme, SOD); and (2) limiting LDL cholesterol oxidation in blood (Ferrari 2001).

53.2.2 Aging Impairs Cardiac and Vascular Functions

Health centenarians' studies have interestingly revealed that in successful aging cardiovascular control is essential, since they have higher HDL cholesterol levels and lower blood pressure than common elderly people (Morley 2004; Zyczkowska and others 2004), and their children have substantially reduced risks of diabetes, hypertension, and heart disease (Perls and Terry 2003).

Recently, it was observed that aging humans have increased levels of endothelin-1 (ET-1, a 21-residue vasoconstrictor peptide), a risk factor for high blood pressure, but execution of aerobic exercises programs reduced ET-1 and normalizes blood pressure (Maeda and others 2003). Corder and others (2001) reported that red wines strongly

inhibited ET-1 synthesis. Aging and hypertension have impairing effects on endothelial-dependent relaxing capacities, decreasing blood flow to important target organs, such as brain (Hongo and others 1998). Aged rats had lowered endothelial vasodilation capacity due to cycloxigenase (COX-2) activation and thromboxaneA_2 releasing (Heymes and others 2000). Reversion of vasodilation impairment is one important pharmacological effect of *G. biloba* and polyphenolics in neuroprotection.

53.2.3 Metal-Chelating Activities

In Alzheimer's disease (AD), massive iron loading is responsible for neuronal damage though Fenton-derived DNA oxidation and (β-amyloid formation (Smith and others 2000). Iron overload is also implicated in autoimmunity disorders and brain damage in autism, where lipoic acid supplementation seems to be beneficial (Padhye 2003). Clioquinol, a metal chelator that cross blood brain barrier, has been successfully used to treat AD (Barnham and others 2004). Natural polyphenolics are able to chelate metals. Quercetin, rutin, catechins, sesamol, caffeic, ferulic, and tannic acids present high antioxidant activities and metal-chelating properties (Chen and Ahn 1998; Lopes and others 1999). Myricetin and quercetin flavonoids are best copper and iron chelators (Mira and others 2002). However, iron deficiency, found in aging, anemia and vitamin B_6 deficiency, also seriously compromises mitochondrial functions contributing to neuronal death (Atamna and others 2002). Table 53.2, summarizes important antiaging functional foods.

53.3 NONSPECIFIC ANTIAGING MECHANISMS

53.3.1 Preventing Cataract and Eye Macular Degeneration (MD)

Macular pigment is constituted by vitamin A, lutein, and zeaxanthin, carotenoids that protect eye by means of ultraviolet light filtration, free radical scavenging, and protection of cell membrane lipids (Moeller and others 2000; Olson 1999). Other carotenoids such as lycopene and β-criptoxantine also quench ROS, especially singlet oxygen, decreasing the risk of MD (Olson 1999). Riboflavin, vitamins C and E can also lower cataract's risk, whereas iron has opposite effects (Bunce 1993; Garner and others 1999).

A case-control study in Burjassot (Spain) revealed that high plasma vitamin C levels ($>$49 μmol/L) were associated with 64% reduction of cataract risk, although intermediary tomato lycopene levels were also associated with decreased disease risk (Valero and others 2002).

The dietary intake of carotenoids, especially lutein and zeaxanthin found in spinach, broccoli, and eggs, was strongly related with lowered risk of cataract and MD (Moeller and others 2000); the *Nurses' Health Study* confirmed these results, although reported a lack of association regarding cataract and dietary intake of other carotenoids (α-carotene, β-carotene, lycopene, and β-criptoxantine) (Chasan-Taber and others 1999). However, Lyle and others (1999) did not find inverse association between carotenoids and risk of cataract, whereas tocopherol has been protective against cataract. Dietary intake of three or more portions of fruit was inversely associated with MD risk (relative risk = 0.64) in comparison to the low consumption group (Cho and others 2004).

TABLE 53.2 Antiaging Biological Mechanisms and Representative Classes of Functional Food Bioactive Compounds.

Mechanisms	Bioactive Compounds	Food Source
Antioxidants	Flavonoids (apigenin, kaempferol, luteolin, myricetin, quercetin, lycopene)	Onion, garlic, tomato, fruits, and vegetables
	Polyphenols	Grapes (juices), wines, berries, apples, cocoa and chocolate, eggplant, teas, etc.
	Curcuminoids	Turmeric
	Monounsaturated fatty acids	Oils and seeds
	Phytosterols (genistein & daidzein)	Soybean
	Tocopherols	Oils and seeds
Antiapoptotic agents	Ascorbic acid	Citrus and other fruits *Ginkgo biloba*
	Egb761 extract (quercetin, kaempferol, isorhamnetin, and bilobalide, a terpene lactone)	Polyphenol rich foods
	Gallic acid	
Metal chelators	Phenols, polyphenols, and their acids (quercetin, rutin, catechins, sesamol, caffeic, ferulic, and tannic acids)	Grapes and wine
Proapoptotic agents	Artellipin C	Brazilian propolis
	Butyrate	Vegetable fibers
	Catechins	Tea polyphenols
	Genistein	Soy
	Indol-3-carbinol	Cruciferae vegetables (broccoli)
	Isoprenoids, terpenoids, and tocotrienols	Vegetable oils (olive oil), nuts (Brazil nuts, cashew nuts, almonds, etc.), and seeds
	Isothiocyanates	Cruciferae
	Fish oils	Fish oil
	Retinoids (vitamin A-related)	Vitamin A rich foods (oils, dark green leaf vegetables, and fruits)
	Polyphenols	Persimmon (*Diospyros kaki*), green teas, wine, berries, and purple fruit
	Protopanaxadiol	Metabolites from ginsenosides (Rb1/Rb2/Rc)
	Organosulfur compounds	Garlic and onion
Mitochondrial stabilizers	Carnosine (β-alanyl-L-histidine)	Muscle foods
	Coenzyme Q_{10} (ubiquinone)	Soy oil, colza seed oil, mackarel fish, sesame seed oil, meat, peanut, pork meat, fish filet, chicken, and nuts
	Melatonin	*Scutellaria biacalensis* (Huang-qin), *Hypericum perfuratum* (St. John's wort), fever few, mustard, and fenugreek seeds
	Lipoic acid	Meat, liver, and heart
	Nicotinamide	Meats, grains, beans, fish, milk, eggs, seeds, vegetables
	n-3 fatty acids	Fish (tuna, mackerel, salmon), canola (rapeseed) and flax-seed oils, flaxseed, and nuts.
	Tocopherol	Oils (olive) and seeds

53.3.2 Cardiovascular Protection

Scavenging free radicals produced during ischemic conditions constitute one of the most important cardiovascular benefits of antioxidant phytochemicals, vitamins, and minerals in foods (Hu and others 1998; Ferrari 1998; Chopra and others 1999; Block and others 2001; Cui and others 2002; Couture 2002; Ferrari 2004).

Blood cholesterol lowering effects represent another important mechanism to protect against cardiovascular diseases. Vegetable and fruit fibers (with pectin), and garlic have hypocholesterolemic effects in humans through partially inhibition of absorption than suppression of hepatic cholesterol synthesis by blocking of hydroxymethylglutaryl-CoA reductase enzyme (Lampe 1999).

Antioxidant vitamins, whole grains, and phytochemicals also protect vascular systems in heart and brain against homocysteine, and independent vascular risk factor (Broekmans and others 2000; McKeown and Jacques 2001; Matson 2003).

Intake of flavonoids, from apples and onion, has been associated with decreased cardiovascular mortality (Knekt and others 1996). Within the cardiovascular protective mechanisms of flavonoids, inhibition of platelet aggregation, increasing of nitric oxide synthesis, and lowering of superoxide production seems to be important (Freedman and others 2001). Ginseng increases antioxidant SOD expression in vitro (Kim and others 1996), induces vascular relaxation and hypotensive effects (Li and others 2001; Couture 2002), which can benefit both cardiac and neurovascular domains. Cardiovascular protective functional foods are summarized in Table 53.3.

TABLE 53.3 Protective Cardiovascular Mechanisms of Functional Foods.

Biological Mechanism	Bioactive Compound(s)	Food Sources
Decreasing of blood cholesterol	Tocopherols, n-3 fatty acids, phytosterols and others	Almonds and nuts (cashew, Brazil)
	Organosulfur compounds	Garlic
	Phytosterols	Margarine
	Omega-3	Fish oil
	Fiber and phytochemicals	Oat cereal
	Genistein and daidzein	Soy
	Eritadenine	Shiitake mushrooms
Inhibition of LDL cholesterol oxidation	Carotenoids	Green leafy vegetables, fruits, and palm oils
	Lycopene-rich foods	Tomato, tomato juice, spaghetti sauce, and tomato oleoresin
	Polyphenolics and monounsaturated fatty acids	Extra-virgin olive oil
	Tea polyphenolics	Green tea
Decreasing blood pressure	Ascorbic acid	Citrus fruits
	Ginsenosides	*Panax Ginseng*
	Quercetin	Onion and garlic
	Tea polyphenols (theaflavins)	Green and black teas
	Unknown (polyphenolics?)	*Psidium guajava* (guava leaves)
Avoiding ischemic-hypoxic myocardial injuries	Curcumin	*Curcuma longa* (turmeric)
	Unknown (polyphenolics?)	Grapes and propolis
Decreasing homocystein	Folate	Fruits, grains, and vegetables
	Vitamin C	Citrus fruits and vegetables
	Vitamin E	Nuts, seeds, and oils

53.3.3 Neuroprotection by Herbs and Functional Foods

Considerable evidence has pointed out that higher intake of vegetables, fruits, and seeds rich in vitamin C, carotenoids and vitamin E are positively associated with better cognitive function and decreased risk of dementia in the elderly (Bates and others 2002; Berr 2000; Youdim and Joseph 2001). An important contribution from the Rotterdam study is that people with higher intake of vitamin C, vitamin E, beta-carotene, and flavonoids had diminished the risk of AD (Engelhart and others 2002).

The pharmacological properties *G. biloba* comprise blood flow enhancing, scavenging of ROS, inhibition of platelet aggregation, protection of striatal dopaminergic system, and inhibition of monoamine oxidase (Bastianetto and others 2000; Louajri and others 2001; Youdim and Joseph 2001). Efective and commonly used in Germany (Wagner 1999; Ernst 2002), *G. biloba* can induce brain hemorrhage as side effect (Vale 1998). Bilobalide a *G. biloba* compound, protected against both ischemic-induced and glutamate-induced neuronal cell deaths (Chandrasekaran and others 2003). Although ginseng has improved memory performance in animals (Youdim and Joseph 2001), it did not enhance brain performance in humans (Ernst 2002). Omega-3 fatty acids (Song and Horrobin 2004) can improve multiple sclerosis pathogenesis (Mayer 1999). Vitamin E (Ortega and Horrobin 2002), carnosine (Hipkiss 2000; Holliday and McFarland 2000), tea polyphenols (Skrzydlewska and others 2002), broccoli isothiocyanates, lipoic acid (Drukarch and others 2001), and tomato (Suganuma and others 2002) can also protect against neurodegeneration. By preventing homocysteine overload, slowing down its oxidative potential, folate can efficiently repair DNA and maintain neuronal integrity, which means decreased risk of neurodegeneration (Mattson 2003; Morley 2004).

53.3.4 Anticancer Activities

Anticancer functional foods can induce (Ferrari and Torres 2003; Kelloff and others 2000): (1) apoptosis of cancer cells; (2) antioxidant protection of DNA; (3) decreasing of oxidative stress and inflammation; and (4) antiangiogenesis.

Anticancer foods and their protective mechanisms are listed in Table 53.2 and above-mentioned.

53.4 FUNCTIONAL FOODS AND HERBS: TOWARDS A HEALTHY DIET

53.4.1 Fish, Fish Oil, and Olive Oil

Fish and fish oils can be beneficial to decrease cancer's risk. In a rat model of azoxymethane-induced colon carcinogenesis, intake of fish oil increased cell proliferation, and apoptosis of colon cells, mechanisms that could avoid colon carcinogenesis (Chang and others 1998). Fish oil, rich in n-3 fatty acids, is responsible for lower degree of cellular rectal proliferation (Biasco and Paganelli 1999). Myocardial infarction was partially reversed by fish oil intake, which improved mitochondrial respiratory functions, partially abrogating lipid peroxidation, lactate production, calcium release, and phospholipase activities (Padma and Devi 2002).

In azoxymethane-induced colon carcinogenesis, rats fed an olive oil rich diet had less aberrant crypt foci and prostaglandin E_2 levels than carcinogen-treated group (Bártoli and others 2000). Olive oil also increases nitric oxide production and decreases prostaglandin synthase-2 in resident macrophages (Moreno and others 2001). Higher dietary intake of olive oil and olive oil cooked vegetables strongly decreased the risk for rheumatoid arthritis in a Greek population (Linos and others 1999). However, Pedersen and others (2000) reported that olive oil increased LDL cholesterol in humans. Notwithstanding, fish oil intake has adverse health effects. Its consumption can increase the risk of bleeding disorders and worsen *Listeria monocytogenes* liver and spleen infections (Irons and others 2003).

53.4.2 Soy Foods: A Look to the Orient

In a mice model of carcinogenesis, dietary intake of soy foods reduced tumor cell proliferation, increased apoptosis, and reduced microvessel density, effects associated with decreased levels of insulin-like growth factor-I, an angiogenic protein (Zhou and others 1999). Soy food and phytoestrogens consumption is associated with many beneficial actions such as antioxidant activities (Wiseman 1996), induction of breast cancer cells apoptosis (Katdare and others 1999), and decreased toxicity of xenobiotic metabolites (Kelloff and others 2000; Xu and others 2000). High intake of soy foods during adolescence was inversely associated with breast cancer risk with a lowering of 65% in risk of this cancer (Shu and others 2001). Unfortunately, soy genistein appears to favor *C. albicans* growth (Yazdanyar and others 2001), a fact that deserves more research and attention from doctors and nutritionists.

53.4.3 Cocoa and Chocolate

Cocoa and dark chocolate have high antioxidant activity, protects LDL from oxidation and decrease platelet aggregation, protecting against heart and vascular diseases (Kondo and others 1996; Rein and others 2000; Richelle and others 2001; Wan and others 2001). These foods can protect genome against mutations (Yamagishi and others 2001).

53.4.4 Calcium and Vitamin A: New Functions for "Old" Nutrients?

Calcium is currently being tested as antineoplastic drug, once it diminishes hyperproliferation of colon cancer cells, decreasing cancer (Lipkin 1999; Kelloff and others 2000). Calcium inhibits ornithine decarboxilase activity, decreases the mutation rate of *ras* (a gene involved in proliferative responses), and promotes the formation of insoluble complexes with bile and fatty acids, decreasing proliferative and irritative effects on intestine (Lipkin 1999; Ferrari and Torres 2002).

Originary from vitamin A or synthesized by human body, retinoids can modulate cell proliferation, differentiation, and apoptosis, decreasing carcinogenesis, effects controlled by six different human nuclear retinoid receptors, grouped as retinoic acid receptors (RAR) and retinoid X receptors (RXR) (Lippman and Lotan 2000). In a rat experimental model of hepatocarcinogenesis, vitamin A and *all trans* and *9-cis* retinoic acids inhibit cell proliferation and oncogenesis (Silveira and others 2001), confirming the anticarcinogenic role of carotenoids and vitamin A in human breast cancer (Zhang and others 1999).

53.4.5 Curcumin (Diferuloylmethane)

Curcumin from turmeric (*Curcuma longa*), an Indian spice very used in oriental culinary, is able to inhibit nuclear factor-kappa-beta (NFκB) activation (Singh and Aggarwall 1995), which is associated with suppression of cell proliferation including inflammatory processes. Curcumin induced apoptosis of promyelocity leukemia HL-60 cells, an effect mediated by increasing intracellular ROS and partial inhibition of Bcl-2 protein (Kuo and others 1996). Antiinflammatory effects (mediated by inhibition of cycloxigenase-2, which is involved in prostaglandin synthesis), xenobiotic detoxifying mechanisms and induction of colon cancer cells apoptosis also constitutes biological properties of curcumin (Manson and others 2000). It also helps to metabolize carcinogens by activation of quinone reductase, an enzyme from cytochrome P450 complex (Dinkova-Kostova and Talalay 1999), and induces expression of the potent antioxidant enzyme heme-oxygenase (Scapagnini and others 2002).

53.4.6 Propolis: An Aid from Bees?

Propolis was used by ancient Egyptians to embalm mummies and has many bioactive compounds, such as flavonoids (flavones, flavonols, flavonones), caffeic acid phenetyl ester (CAPE), terpenoids, and other aromatics, fatty acids, phenols, aminoacids, vitamins (A, B1, B2, B6, C, and E), and minerals (Mn, Cu, Ca, Al, Si, Ni, Zn, and Cr), which explains its antibiotic, antitrypanosomal, antiviral, antimutagenic, and immunomodulatory properties, justifying its widespread and current use in dermatological and periodontal products (Pereira and others 2002). Brazilian propolis at low concentrations inhibited growth and survival of *C. albicans* and *C. tropicalis* (Sforcin and others 2001). CAPE is a potent inhibitor of NFκB and is able to induce apoptosis in HL-60 cells, an effect mediated by increasing intracellular ROS and decreasing glutathione (GSH) (Natarajan and others 1996; Chen and others 1998). In an experimental model of ferric nitrilotriacetate-induced lung carcinogenesis, Kimoto and others (1998) reported that Brazilian propolis extract and artepillin C decreased lipid and DNA peroxidation, blocking cancer progression. Propolis block COX-2 synthesis (Michaluart and others 1999) and increase SOD synthesis (Sforcin and others 1995), performing antiinflammatory and antioxidant effects, respectively.

53.4.7 Intake of Fruit and Vegetable Fibers Protects Against Gastrointestinal Cancers

Dietary fiber decreases intestinal time bulk transit, detoxify bile acids, and has other beneficial effects, protecting not only intestine, but also decreasing the risk of esophageal and gastric cardia adenocarcinomas (Roth and Mobarhan 2001).

Butyrate, a short-chain fatty acid from vegetable fibers, induces apoptosis and decreases cell proliferation, inhibiting colon cancer cells (Biasco and Paganelli 1999; Chapkin and others 2000; Kelloff and others 2000). Found in fibers and milk fat, butyric acid and tributyrin were able to induce apoptosis of human liver cancer cells (Watkins and others 1999).

53.4.8 Eat Tomato, Broccolis, and Garlic . . . Daily!

Lycopene, a tomato carotenoid, was not a potent growth inhibitor of human prostate carcinoma cells, but in association with α-tocopherol both decreased viability of tumor

cells by 90% (Pastori and others 1998). Dietary intake of tomato is very important to decrease risk of prostate cancer and possibly other tumors (Weisburger 1999).

Garlic, an ancient popular "medicinal food", is constituted by many sulfur-rich biomolecules, which confers high antioxidant activity (Borek 2001). In spite of a slight effect on platelet aggregation, garlic extracts can potently affect platelet adhesion to fibrinogen, collagen, and von Willebrand factor (Steiner and Li 2001). Oil-soluble garlic has potent antiproliferative and anticarcinogenic effects modulated by inhibitory effect on protein kinase involved in cell cycle progression and induction of apoptosis (Knowles and Millner 2001). In dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis, administration of S-allyl-cysteine, a water-soluble garlic compound, prevented carcinogenesis and significantly increased GSH, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) levels (Balasenthil and Nagini 2002). In a rat model of streptozotocin-induced diabetes, Campos and others (2003) observed that onion feeding lowered both hyperglycemia and SOD levels, without increase lipid peroxidation, whereas HDL cholesterol levels consistently increased. Unfortunately, garlic is not exempt from side effects. It enhances effectiveness of anticoagulant warfarin, increasing bleeding occurrence (DasGupta 2003).

When glucosinolates from broccoli suffer hydrolysis in human gut they yield isothiocyanates, indol-3-carbinol and sulphoraphane, a group of bioactive compounds able to increase hepatic cytochrome P_{450} xenobiotic detoxification, induce antioxidant response-element cytoprotection, decrease the risk of hormone-dependent cancers, and trigger growth inhibition and apoptosis of breast, colon, prostate cancer cells (Chen and others 1998; Kelloff and others 2000; Ferrari and Torres 2002; Xiao and Singh 2002; Keck and Finley 2004). It is important to note that many glucosinolates are also environmental carcinogens (Ames 2004).

53.4.9 Grapes and Wine

Dietary intake of grapes and wine polyphenols results in potent antioxidant and antiinflammatory cell activities, affording strong neuroprotection (Youdim and Joseph 2001). Wine has many anticarcinogenic effects mediated by suppression of tumor growth and inhibition of transformed cell proliferation and induction of apoptosis, actions attributed to quercetin (Wei and others 1994), cathechin (Stavric 1994), and resveratrol (Soleas and others 2000). Resveratrol was associated with inhibition of androgen-receptor activity for hormones in prostatic tumor cells (Mitchell and others 1999). Potent antioxidant activities, suppressive actions on phase I xenobiotic enzymes and triggering of phase II metabolism, inhibition of prostaglandin synthesis and NFκB pathway, induction of cell cycle arrest, estrogen-receptor mediated activities, and antiangiogenic activities constitute other pharmacological mechanisms of resveratrol (Brakenhielm and others 2001; Savouret and Quesne 2002).

53.4.10 Neuroprotective Effects of Teas (*Camellia sinensis*) and Coffees (*Coffea arabica*)

Antioxidant activity of coffee can prevent oxidative DNA damage (Vieira and others 1999). Coffee has also higher antioxidant activity in vitro, measured as lag-time to LDL oxidation, as compared to cocoa, green tea, black tea, and herbal tea (Richelle and others 2001). Several epidemiological studies had reported that coffee intake is inversely associated with risk for Parkinson's disease and AD (Ross and others 2000; Maia 2001).

Blocking A_2A receptors from nigrostriatal dopaminergic neurons by caffeine is the suggested protective mechanism of coffee (Schwarzschild and others 2003). Beyond the recognized negative effects of caffeine on blood pressure, heavy coffee consumption seems to increase plasmatic levels of homocysteine (Urgert and others 2000). Testing if coffee drinking can perform neuroprotection is an important achievement, once its daily consumption is very common around the world.

Tea polyphenols (epicatechins, catechins, and thearubigin) are potent antioxidants and decrease the risk of oral and gastrointestinal cancers (Yang and others 1998; Weisburger 1999; Ferrari and Torres 2003). Administration of green tea polyphenols (5 g/L) to interleukin-2-deficient rats (a model of autoimmunity) decreased inflammatory biomarkers (IFN-γ e TNF-α) and severity of colitis (Varilek and others 2001). Green tea consumption is also associated with low incidence of cardiovascular disease in the Orient by means of decreasing cholesterol oxidation and atherosclerosis (Miura and others 2001). Tea also blocked angiogenesis slowing down the metastatic capacity of tumors (Cao and Cao 1999).

53.5 CONCLUSION

Regular daily intakes of functional foods and adoption of a healthy lifestyle, which incorporates regular practice of physical activities (Maeda and others 2003; Mahoney and others 2002) improves health and reduces the burden of aging.

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54

Functional Foods and Gastrointestinal Disorders

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54.1 Introduction 154
54.2 Historical Context 154
54.3 Mechanisms of Action of Probiotics 155
54.3.1 Defense Against Infection and Inflammation 155
54.3.1.1 Activities Within the Lumen 155
54.3.1.2 Activities at the Epithelial Surface 156
54.3.1.3 Activities at the Mucosal Immune System 157
54.3.2 Metabolic Effects of Probiotics 161
54.3.3 Anticarcinogenic Effects 163
54.4 Effects of Probiotics in Other Disorders 163
54.4.1 Effects of Probiotics in Gastrointestinal Disorders 163
54.4.1.1 Irritable Bowel Syndrome 163
54.4.1.2 Clostridium Difficile-Associated Intestinal Disease 163
54.4.1.3 Pouchitis 166
54.4.1.4 Lactose Intolerance 166
54.4.2 Effects of Probiotics in Extraintestinal Disorders 166
54.4.2.1 Allergies 166
54.4.2.2 Old Age 167
54.4.2.3 Hepatic Encephalopathy 168
54.4.2.4 Sucrase-Isomaltase Deficiency 168
54.4.2.5 Hypercholesterolaemia 168
References 168

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54.1 INTRODUCTION

Gastroenterologists seldom think about food consumption outside the context of excesses (obesity) and deficits (malabsorption and malnutrition). However, the clinical perception of food quality and quantity is changing as physicians must contend with emerging evidence for functional foods. Functional foods and food supplements are operationally defined as foods with health benefits beyond their nutritional content (Shanahan and McCarthy 2000). Consumers are increasingly aware of food labels and economists have predicted that the consumer will soon demand that all foods have a functional content (McCarthy and others 2004). Terms such as pharmafood, vitafood, medical food, nutraceutical, and designer food are now commonplace as the distinction between a food and a drug becomes blurred. Although some clinicians dismiss the notion of functional foods as being in the realm of alternative medicine or food faddism, there is sound scientific evidence in support of a direct health benefit from certain food supplements. Examples include the supplementation of drinking water with fluoride to prevent dental caries, the use of folate to prevent neural tube defects and cancer and the exploitation of oral tolerance within the gastrointestinal tract to treat or prevent autoimmunity by feeding putative self-antigens. Perhaps the most impressive evidence for functional foods in treating or preventing intestinal disease is in the area of therapeutic manipulation of the gut flora with probiotics and our focus in this chapter is on mechanisms of probiotic action in gastrointestinal disorders.

54.2 HISTORICAL CONTEXT

In 1907 the Russian Nobel Prize Laureate Elie Metchnikoff first hypothesized that a high concentration of lactobacilli in intestinal flora were important for health and longevity in humans (Metchnikoff 1907). The term "probiotic" dates to 1954 when Ferdinand Vergin compared the harmful effects of antibiotics on the flora and the beneficial effects of certain bacteria (Vergin 1954). In 1965, the term probiotic was used to describe any substance or organism that contributes to intestinal microbial balance (Lilly and Stillwell 1965). Fuller subsequently stressed the benefits of a live microbial feed supplement in treating human disease (Fuller 1991). Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Isolauri and others 2002). For the purpose of this chapter probiotics are operationally defined as commensal organisms that can be harnassed for health benefit.

Most probiotics belong to a large group of bacteria empirically designated as lactic acid bacteria (*lactobacilli*, *streptococci*, *bifidobacteria*). These are important components of the human gastrointestinal microflora where they exist as harmless commensals. Lactic acid is the principal end product of metabolism for all these species. Other probiotics include microbes such as yeast (e.g., *Saccharomyces boulardii*) and entirely unrelated bacteria (e.g., *E. coli nissle*). While some probiotics may be species specific it remains to be clarified if probiotics must be of human origin. They must be both acid and bile resistant and have the ability to sustain metabolic activity within the luminal flora, where ideally they should survive, if not persist for the long term.

54.3 MECHANISMS OF ACTION OF PROBIOTICS

Many diverse theories have been proposed for the mechanisms of action of probiotics. However a definitive understanding of the exact mechanisms eludes us. It is likely however that these mechanisms are multifactorial and both strain and host specific. It would be simplistic to assume that probiotics are generic, behaving the same way and that one strain is suitable for all patients.

54.3.1 Defense Against Infection and Inflammation

The role of probiotics in enteric infections is summarized in the Cochrane review (Allen and others 2003). Activities of probiotics appear to take place at least three levels, which are not necessarily mutually exclusive, the luminal environment, the epithelial surface and the mucosal immune system, as shown in Figure 54.1.

54.3.1.1 Activities Within the Lumen

Competition for Substrates. Probiotics may affect the growth and function of pathogenic bacteria by competing with them for nutrients in the gut. *Bifidobacterium longum* has several high-affinity oligosaccharide transporters, including those for more than 80 oligosaccharides in breast milk. This could be the mechanism which would favor its presence and competition for substrates in the breastfeeding infant (Schnell and others 2002). However, *in vivo* evidence is lacking and this theory might not be true due to the abundance of nutrients in the gut.

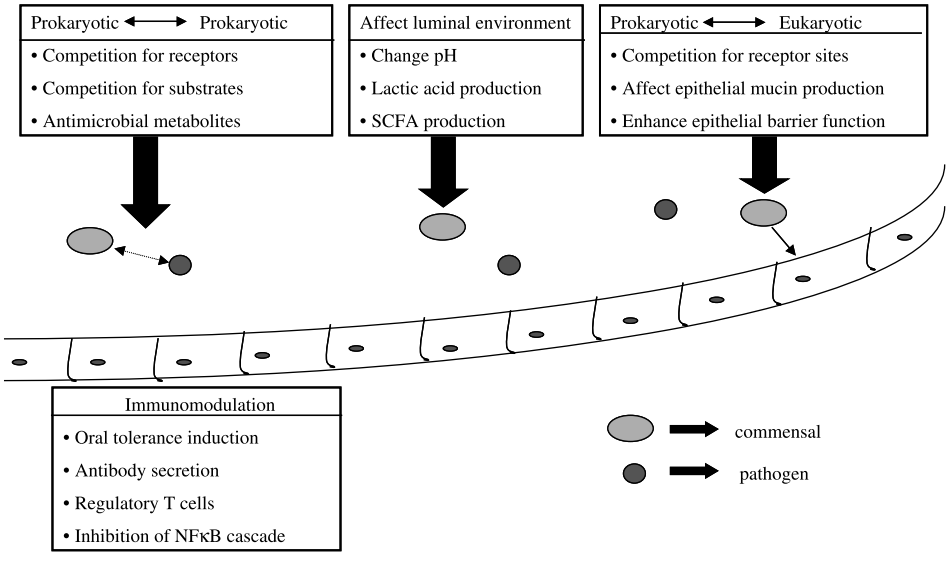

Figure 54.1 *Conceptual overview of examples of potential mechanisms of actions of probiotics in the Gastrointestinal tract.*

Generation of a Restrictive Environment. Probiotics may also affect the colonic environment making it less conducive to growth of certain pathogenic components of the flora. *Lactobacillus* in particular has been shown to reduce colonic lumen pH through production of short-chain fatty acids (Johansson 1993) and lactic acid. This is the major mechanism used by lactic acid bacteria to inhibit growth of several anaerobes. Production of hydrogen sulfide and effects on the oxidation-reduction potential by certain species of the flora have also been suggested as possible mechanisms.

Production of Antimicrobial Metabolites. Another important quality of probiotics in enhancing host defense is their ability to secrete antimicrobial substances that inhibit the growth of other organisms for example, *E. coli*, *C. difficile*, and *salmonella* (Aiba 1998; Fekety and Shah 1993; McFarland and others 1989). Other antimicrobial factors such as lactic acid and bacteriocins are produced mainly by lactic-acid bacteria (Flynn and others 2002). Bacteriocins are ribosomally synthesized proteins (Moll and others 1999). They show bacteriocidal activity against species that are closely related to the bacteria that produce them (Tagg and others 1976). Biochemical and genetic characteristics divide bacteriocins into three different classes: (1) lantibiotics, (2) nonlantibiotics, which are both small, membrane-active and heat-stable, and (3) large heat-labile proteins (Moll and others 1999). A number of *lactobacillus* bacteriocins and their genetic determinants have been described. Their inhibitory spectrum varies greatly most inhibit other *lactobacilli* or related Gram-positive bacteria, whereas some are active against a wide range of Gram-positive or Gram-negative bacteria as well as yeasts and moulds (Nemcova 1997). It is likely that production of these proteins plays a role in the survival and persistence of these organisms in the gastrointestinal tract.

The production of lactic acid has been implicated in the ability of certain probiotics to overcome pathogenic bacteria and thus exert a beneficial effect on the host. This has been most notable in studies looking at the effect of probiotics on *Helicobacter pylori* infection. Probiotics were able to inhibit the growth of *H. pylori in vitro* and in human and murine models (Aiba 1998; Michetti and others 1999). Both studies established that this effect is due to the production of large quantities of lactic acid as the inhibitory effect could be reproduced after incubation of *H. pylori* with lactic acid. They found that lactic acid has bacteriocidal activity and also prevents *H. pylori* adherence to gastric mucosa cells. The outer membrane of Gram-negative bacteria becomes permeable rendering it susceptible to other antimicrobial substances. A further study of the effect of a *L. acidophilus* strain on *H. pylori* activity *in vitro* showed that an autolytic process occurs that is linked to a proteinaceous compound released after cell lysis (Lorca and others 2001).

54.3.1.2 Activities at the Epithelial Surface. Activities of probiotics probably mimic the normal gut flora. Probiotics influence the mucosal epithelium mainly by competing for receptor sites and adherence.

Competition for Receptor Sites. Some commensal bacteria prevent adhesion of pathogens to receptor sites mostly by spatial hindrance due to their size and number and by blocking specific receptors. One study showed that probiotics have effects on pathogenic bacterial binding in the colon. IL-10 deficient mice exhibit increased mucosal adherence or invasion of aerobic bacteria in the colon that precedes the development of colitis. This is coupled with a decrease in colonic luminal lactobacillus species. However restoration

of lactobacillus species levels to normal reduces the concentration of adherent and translocated bacteria and thus attenuates the colitis (Madsen and others 1999).

It has also been proposed that probiotics release pathogen-inhibitory substances that inhibit bacterial translocation. *Lactobacillus GG* was found to have a direct effect on enterocytes that results in diminished passage of pathogens across the epithelial monolayer by upregulating the epithelial barrier mechanism (Mattar and others 2001). *L. GG* also binds to cell surface receptors on enterocytes and thus competes with pathogenic bacteria enterocyte attachment.

Steric hindrance of pathogen receptors was demonstrated after several heat inactivated strains of *lactobacillus* species were able to adhere to Caco-2 (a human colon cancer epithelial cell line) cell cultures and inhibit the adhesion of pathogenic *E. coli* and *S. typhimurium (*Chauviere and others 1992).

Competition for Adherence. Probiotics have been shown to directly and indirectly inhibit adherence of organisms to intestinal epithelial cells. Their effects on intestinal mucins play an important part. Mucins are high molecular weight glycoproteins synthesized and secreted by epithelial cells. They diminish the ability of enteric bacteria to bind to mucosal epithelial cells. In fact they are a component of the epithelial cell's innate immune response to invasive bacteria; MUC2 and MUC3 being the predominant ileocolonic mucin genes.

A study on the *in vitro* effects of probiotics on epithelial cell function showed that probiotic agents, such as *L. plantarum 299v*, which are able to bind to epithelial cells *in vitro* and colonize the intestinal tract *in vivo* (Johansson 1993), induce epithelial cells to secrete mucins that diminish enteric pathogens binding to mucosal epithelial cells (Mack and others 1999).

Mucins isolated from intestinal tracts of animals have been shown to inhibit *in vitro* adherence of pathogenic bacteria such as *E. coli* (Mack and Sherman 1991; Forstner 1994; Smith 1995). This interruption of the enteropathogen adherence to intestinal epithelial cells could provide therapeutic benefit to the host. However the mechanism by which *Lactobacilli* may cause increased mucin gene transcription remains to be determined.

Other research has shown how certain microorganisms are able to modify the cellular carbohydrate pattern thereby promoting colonization of specific beneficial bacteria. *Bacteroides thetaiotaomicron strain VPI-5482* changes a specific galactosylation pathway in HT-29-MTX intestinal epithelial cell line through a heat labile soluble factor (Freitas and others 2001). The probiotic compound *VSL#3* has a direct effect in enhancing barrier integrity in the IL-10 K.O. mouse. This is likely to be secondary to a proteinaceous soluble factor that is secreted by the bacteria found in *VSL#3*. There is a similar effect on human epithelial barrier function (Madsen and others 2001).

54.3.1.3 Activities at the Mucosal Immune System

Immunomodulation. The gut immune response to microorganisms relies on both innate and acquired components of immunity. The innate response is a primitive but highly conserved immune response which discriminates between pathogens and commensals using preformed receptors known as toll-like receptors. This allows immediate recognition of bacteria and the host can respond accordingly by activating innate immune cells. The more specific component of the immune system is the adaptive response. This is responsible for formation of memory. Cells of the adaptive immune response recognize and

recall antigens using antigen specific receptors (T-cell receptors and immunoglobulins). The complex interaction between the innate and adaptive immune systems protects the host against pathogens, which preserves intestinal mucosal function. Precise regulatory mechanisms exist that ensure appropriate inflammatory reactions and that nonpathogenic commensals are tolerated at local and systemic levels. It has been suggested that probiotics may mimic normal flora through modulation of this host immune response. They educate and prime the immune response.

Probiotics have been shown to increase the synthesis of antibodies to microbial pathogens, especially secretory IgA (Kaila and others 1992). Certain probiotic bacteria induce specific immune responses and hyporesponsiveness to commensals. This tolerance induction matures the gastrointestinal associated lymphoid tissue such that it is ready to produce IgA in response to pathogen antigenic stimulus. In support of this, defective oral tolerance to commensals and ingested antigens has been shown in germ-free animal models (Faria and Weiner 1999).

Probiotics may also exert their effects at the level of regulatory T-cells. Many animal models have been used to investigate the balance between effector and regulatory T-cells in the inflammatory processes of the gut. Some groups have demonstrated that regulatory T-cells exist that control mucosal immunity to the enteric flora (Kronenberg and Cheroutre 2000; Shanahan 2000). It has been demonstrated in murine models that the mucosal response to enteric antigens is linked with a predominance of T helper cells of the Th2 and Th3 types. The Th2 response produces anti-inflammatory cytokines (IL-4, IL-10), which promote the secretion of IgA and Th3 produces TGF-β. Both of these responses are critical for the development of oral tolerance. Probiotics may also influence the development and activity of these regulatory T-cells. This mechanism may account for their apparent clinical efficacy in disorders involving Th1 immunological processes, such as inflammatory bowel disease.

The mechanisms used by the immune system to recognize pathogens and commensals are still not well understood. Dendritic cells are involved in antigen presentation to the mucosal immune system and it may be at this level that the bacteria are differentiated. Commensals presented by dendritic cells to mesenteric lymph nodes do not elicit a systemic immune reaction. Dendritic cells can imprison live commensals for days and produce specific IgAs which then prevent these commensals from penetrating the gut mucosa (Macpherson and Uhr 2004).

There is increasing evidence that some of the probiotic effects encountered may not require a whole live bacterium. Bacterial DNA has been successfully used to attenuate murine models of colitis by their potent immunostimulatory effects. It is thought that this attenuation could be due to antiapoptotic mechanisms. These DNA sequences or immunostimulatory sequences (ISS) or CpG motifs are under extensive study (Rachmilewitz and others 2002).

Signaling. The transcription factor NFκB is pivotal to the inflammatory immune response to invasive pathogens (Elewaut 1999). It has been postulated that probiotics could exert an antiinflammatory effect through the inhibition of this pathway.

Certain probiotics exert antiinflammatory actions on epithelial cells by inhibition of NFκB. It has been shown that probiotic DNA downregulated proinflammatory cytokine secretion by attenuation of NFκB pathway in intestinal epithelial cells (Madsen 2002). The counter-regulatory factor to NFκB is IκB and some nonpathogenic bacteria may attenuate proinflammatory responses by delaying its degradation (Neish and others 2000). Recently *Bacteroides thetaiotaomicron*, a nonpathogenic commensal,

TABLE 54.1 Summary of Human Trials of UC.

Probiotic Used	No. of Subjects	Study Type	Results	Reference
E. coli strain Nissle 1917	120	Randomized controlled trial	Patients with active colitis had similar relapse rates compared to patients on mesalazine	Kruis and others 1997
E. coli strain Nissle 1917	116	Randomized controlled trial	As above	Rembacken and others 1999
E. coli strain Nissle 1917	327	Randomized controlled trial	*E. coli* as effective as mesalazine in maintaining remission	Kruis and others 2004
VSL#3	20	Open labeled trial	Maintained remission in 75% of patients	Venturi and others 1999
Saccharomyces boulardii	25	Open labeled trial	68% of patients with a relapse of UC achieved remission when treated with a combination of probiotic and mesalazine	Guslandi and others 2003
Bifidobacterium longum with prebiotic inulin-oligofructose	18	Randomized controlled trial	Short term treatment of active UC resulted in improvement of the full clinical appearance of chronic inflammation	Furrie and others 2005
Balsalazide (5-amino salicylate derivative) with VSL#3	90	Randomized controlled trial	Low-dose balsalazide with a high dose of VSL#3 is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis	Tursi and others 2004
BIFICO – probitic mix	30	Randomized controlled trial	20% in the *BIFICO* group had relapses during 2-month follow-up period, compared with 93.3% in placebo group	Hai-Hong Cui and others 2004

TABLE 54.2 Summary of Trials in CD.

Probiotic Used	No. of Subjects	Study Type	Results	Reference
Saccharomyces boulardii	20	Randomized controlled trial	Decrease in CDAI in probiotic group	Plein and Hotz 1993
Saccharomyces boulardii	32	Randomized controlled trial	Maintenance of remission in treatment group superior as relapse observed in 6.25% of patients receiving probiotic plus mesalazine compared to 37.5% on mesalazine alone	Guslandi and others 2000
Lactobacillus GG	14	Open labeled trial	Increase in gut IgA response	Malin and others 1996
Lactobacillus GG	4	Open labeled trial	Improved intestinal permeability and CDAI	Gupta and others 2000
Lactobacillus GG	45	Randomized controlled trial	No difference seen in rate of recurrence 1 year after surgery between group given probiotic or control	Prantera and others 2002
Lactobacillus GG	5	Randomized controlled trial	No benefit in inducing or maintaining medically induced remission in CD	Schultz and others 2004
E. coli strain Nissle 1917	28	Randomized controlled trial	Remission achieved in patients on probiotics and steroids greater than with steroids alone	Malchow 1997
VSL#3 with antibiotic	40	Randomized controlled trial	Patients with CD had 20% remission when given antibiotic and *VSL#3* compared to 40% in mesalazine treated group	Campieri and others 2000
Lactobacillus salivarius 118	25	Open labeled trial	Reduction of mean CDAI and induction of IgA in patients with relapse	McCarthy and others 2001; 2004

was demonstrated to target the transcriptionally active NFκB subunit RelA and enhance its nuclear export thereby preventing an inflammatory response in colonic epithelial cell lines (Kelly and others 2004). This group also showed how this bacterium exerts the above effect through a PPAR-γ dependent pathway. Peroxisome proliferator activated receptors (PPAR) activation by PPAR ligands not only result in the transcription of antiinflammatory genes but also inhibit NFκB directly. Nonpathogenic bacteria also exert antiinflammatory effects by inhibiting IκB ubiquitination (Neish and others 2000) This would preserve IκB (inhibitory κB) so that NFκB pathway is not activated.

There is evidence from murine studies to suggest that IL-10 is an essential modulator of the regulatory T-cells that control inflammatory responses to intestinal antigens. It suppresses the inflammatory immune response in the gut mucosa by promoting the activity of regulatory T-cells that hold effector Th1 cells in check. Murine models with targeted disruption of the IL-10 gene develop enterocolitis. Administration of IL-10 has provided therapeutic benefit in a human study of inflammatory bowel disease (Van Deventer and others 1997). Experiments have shown that IL-10 can restore tolerance of T-cells to resident intestinal bacteria. A genetically-engineered bacteria to synthesize IL-10 within the intestinal lumen of murine models was administered with good antiinflammatory results (Steidler and others 2000). Other murine studies have looked at the effect of probiotics on enterocolitis and the immune response in IL-10 knockout mice. Our group found that specific strains of both *lactobacillus* and *bifidobacterium* significantly attenuate colitis in this model. They showed that this attenuation of colitis was associated with a reduced ability to produce Th1-type proinflammatory cytokines (TNF-α, IL12) at a mucosal and systemic level whereas TGF-β levels were maintained (McCarthy and others 2003). A summary of human trials of probiotics in inflammatory bowel disease can be seen in Tables 54.1 and 54.2.

54.3.2 Metabolic Effects of Probiotics

Probiotics may exert beneficial effects on the host through their effects on metabolism of dietary elements such as polysaccharides affecting energy intake, absorption, and storage. A seminal study of the effect of recolonising germ-free mice with normal microflora showed that the gut microflora regulates fat storage through promotion of monosaccharide absorption from the gut lumen leading to induction of hepatic lipogenesis, and deposition of triglycerides in adipocytes (Backhed and others 2004).

Probiotics also enhance gut-associated lymphoid tissue responses directly or indirectly through the production of short-chain fatty acids (Bornet 2002*)*. Short-chain fatty acids are the end products of anaerobic bacteria breakdown of carbohydrates in the large bowel. They are readily absorbed by the intestinal mucosa, are relatively high in caloric content, are metabolized by colonocytes, stimulate sodium and water absorption in the colon, and are trophic to the intestinal mucosa. There is increased interest recently in their role in the treatment of inflammatory bowel disease. Their effects have been attributed to the oxidation of these fatty acids in colonocytes and to the ability of butyrate (a SCFA) to induce enzymes promoting mucosal restitution (D'Argenio and Mazzacca 1999). The significance of these acids in immunomodulation needs further consideration.

The ability of probiotics to synthesize beneficial vitamins is speculative at best. However one study found that folate was produced by *bifidobacterium* and *streptococcus thermophilus* and depleted by *lactobacillus* (Crittenden and others 2003). Vitamin K is

TABLE 54.3 Summary of Trials in Cancer.

Bacterial Species	Action	Mechanism of Action	Reference
L. acidophilus, L. gasseri, L. confuses, Streptococcus thermophilus, Bifidobacterium breve, B. longum	Inhibits N′-nitro-N-nitrosoguanidide (MNNG) and 1,2-dimethylhydrazine (DMH)-induced genotoxicity in rat colons	Reduction of DNA damage induced by chemical carcinogens	Pool-Zobel and others 1996
Bifidobacterium longum	Inhibits liver, colon, and mammary tumors induced by food mutagen 2-amino-3-methyl-3H-imidazo (4,5-f) quinoline (IQ) in rats	Inhibition of potential carcrinogens	Reddy and Rivenson 1993
L. acidophilus NCFM and *N-2*	Decrease in bacterial enzyme activity: B-glucuronidase, nitroreductase, azoreductase, in healthy volunteers	Alteration of metabolic activities of intestinal microflora	Goldin and Gorbach 1984
L. acidophilus, B. bifidum	Decreases fecal pH and decrease in proliferative activity in colonic crypts in patients with colonic adenomas	Alteration of physicochemical conditions in the colon	Biasco and others 1991
Lactic acid bacteria	Reduces growth and viability of HT-29 human colon cancer cell line	Production of antitumorigenic compounds	Baricault and others 1995
L. casei Shirota	Inhibits tumor growth and increases survival in mice	Enhancing the immune response by producing cytokines	Matsuzaki 1998
L. casei YIT 9018	Potentiates systemic immune responses that modify T-cell functions in tumor-bearing mice	Enhancing the immune response	Kato and others 1994
B. Longum	Suppresses azoxymethane (AOM)-induced colonic tumor development, effect which is associated with a decrease in the activities of colonic mucosal and tumor ornithine decarboxylase and ras-p21	Effect on host physiology	Reddy 1998

produced by a wide variety of bacteria including *bacteroides*, *bifidobacterium*, *lactobacillus*, *clostridium*, *enterococcus*, and *streptococcus* (Hill 1997). In other studies looking for production of vitamins, no increased production of B complex vitamins was detected. The role of probiotics in the synthesis of vitamins is unlikely to be significant. However they may alter the microflora environment making the absorption of vitamin substances more efficient.

54.3.3 Anticarcinogenic Effects

The anticarcinogenic effects of probiotics are putative, few epidemiological studies exist to support this however there is some indirect evidence in the literature, largely from laboratory studies. In recent years several good animal models have been established to investigate the link between colon cancer and dietary factors, which may be protective, such as lactic acid probiotics (Hirayama 2000; Lee 2000). The precise mechanisms by which lactic acid bacteria may exert an anticarcinogenic effect are unknown but suggestions include: altering the metabolic activities of the intestinal microflora, by changing physicochemical conditions in the colon and host physiology, by binding and degrading potential carcinogens, and by the production of antitumerogenic compounds (Rafter 2003). A summary of trials investigating probiotics in cancer, adapted from work published by Rafter can be seen in Table 54.3.

54.4 EFFECTS OF PROBIOTICS IN OTHER DISORDERS

54.4.1 Effects of Probiotics in Gastrointestinal Disorders

54.4.1.1 Irritable Bowel Syndrome. This is characterized by abdominal cramps or bloating, alternating bowel habit, or excessive flatus without an organic cause. A disruption of the normal flora could result in these symptoms. Low numbers of *lactobacilli* and *bifidobacteria* have been isolated from the faeces of patients with this disorder. It has been suggested that probiotics may restructure the intestinal microflora or its activity (Madden and Hunter 2002). In a recent randomized placebo-controlled trial our group demonstrated the effects of *Bifidobacterium infantis 35624* and *Lactobacillus salivarius UCC4331* on symptom response and cytokine ratio in IBS patients (O'Mahony and others 2001). For all symptoms, with the exception of bowel movement frequency and consistency, those randomized to *B. infantis 35624* experienced a greater reduction in symptom scores. At baseline, patients with IBS demonstrated an abnormal IL-10 to IL-12 ratio, indicative of a proinflammatory, Th-1 state. This ratio was normalized by *B. infantis 35624* feeding alone. It was therefore concluded that *B. infantis 35624* alleviates symptoms in IBS and this is associated with normalization of the ratio of an antiinflammatory to a proinflammatory cytokine response, suggesting an immunomodulatory role for this organism. A review of the literature shows only eight trials in 29 years, six of which show improvement in various symptoms. Most of the trials used *L. plantarum*, solely or in combination. A summary of the trials of probiotics in irritable bowel disease can be seen in Table 54.4.

54.4.1.2 Clostridium Difficile-Associated Intestinal Disease. The role of the probiotic organism in C difficile infection is to restore the colonization resistance of the

TABLE 54.4 Summary of Human Trials of IBS.

Probiotic Used	No. of Subjects	Study Type	Results	Reference
Streptococcus faecium	54	Randomized controlled trial	Improvement of overall condition in 81% compared to 41% in controls	Gade and Thorn 1989
L. plantarum (DSM 9840)	60	Randomized controlled trial	Decrease in pain and flatulence but no effect on bloating	Nobaek and others 2000
Lactobacillus GG	25	Randomized controlled trial	No effect on pain, urgency or bloating. A trend towards more formed bowel motions noticed in patients with diarrhea in those on probiotic	O'Sullivan and O'Morain 2000
L. plantarum 299V (L299V)	40	Randomized controlled trial	All patients (20) on the probiotic reported resolution of their abdominal pain compared to 11 out of 20 in placebo group. 95% on *L299V* reported improvement of all symptoms compared to 15% in placebo group	Niedzielin and others 2001
L. plantarum 299V (L299V)	12	Double-blind, crossover trial	No improvement in symptoms found	Sen and others 2002

VSL#3	25	Randomized controlled trial	No effect on abdominal pain, urgency and gas but decrease in bloating in diarrhea-predominant IBS. No effect in gastrointestinal transit	Kim and others 2003
L. plantarum LP 01 with *B. Breve BR 03*. *Lactobacillus plantarum LP 01* with *Lactobacillus acidophilus LA 02*	70	Randomized controlled trial	Decrease in pain score and symptom severity score in both groups as compared to placebo	Saggioro 2004
Bifidobacterium infantis 35624, *Lactobacillus salivarius UCC4331*	77	Randomized controlled trial	*B. infantis 35624* alleviates symptoms in IBS except for bowel movement frequency and consistency and is associated with normalization of the ratio of an antiinflammatory to a proinflammatory cytokine. *L. salivarius UCC4331* was no better than placebo	O'Mahony and others 2005
Lactobacillus acidophilus	18	Randomized controlled crossover trial	Reduction of symptoms in 50% on probiotic	Halpern and others 1996
Encapsulated Bifidobacterium infantis 35624	291	Randomized controlled crossover trial	Relief of all cardinal symptoms using lower daily encapsulated dose	Whorwell and others 2005 (at press)

normal flora, which has been disrupted by antibiotic therapy (Kyne and Kelly 2001). A number of studies on human subjects have shown promising results (Elmer 2001). The probiotic agents that have been used include *lactobacillus GG*, *bifidobacterium infantis*, and *saccharomyces boulardii* (McFarland and others 1994). Uncontrolled studies showed that small numbers of children and adults with *C. difficile* responded well to treatment with probiotics (Gorbach and others 1987; Biller and others 1995). A prospective randomized placebo-controlled trial of *lactobacillus GG* in combination with standard antibiotics showed that this probiotic was effective in reducing recurrence rates of *C. difficile* and there was subjective improvement in patient symptoms (Pochapin 2000).

The antimicrobial activity of *bifidobacterium infantis* and *lactobacillus salivarius* against *C. difficile* was identified by a Korean group using PCR methods. The probiotic species they used were isolated from healthy infant faeces (Lee and others 2003).

Saccharomyces boulardii is a nonpathogenic yeast that has been shown to protect against antibiotic-associated diarrhoea and recurrent *C. difficile* colitis (Elmer and McFarland 1987; Castagliuolo and others 1996; McFarland and others 1994). This yeast stimulates intestinal immunoglobulin A immune response to *C. difficile* toxin A in mice and enhancing host intestinal immune responses may be an important mechanism for *S. boulardii*-mediated protection against diarrhoeal illnesses (Qamar and others 2001). It has also been postulated that *S. boulardii* protects animals against *C. difficile* intestinal disease through a protease degradation of the toxin receptor on the intestinal mucosa (Castagliuolo and others 1999). Further work to elucidate the exact mechanism of action of probiotics in modifying *C. difficile* inflammatory response is needed.

54.4.1.3 Pouchitis. Probiotics also demonstrate some benefit in pouchitis, a complication occurring in up to 20% of patients with inflammatory bowel disease (IBD) who have undergone pouch formation after bowel resection. VSL#3, a probiotic cocktail of four lactobacilli strains (*L. plantarum*, *L. casei*, *L. acidophilus*, *L. delbrueckii* ssp. *bulgaricus*), three bifidobacteria strains (*B. infantis*, *B. breve*, *B. longum*) and one strain of *Streptococcus salivarius* ssp. has been used in clinical trials of pouchitis with some success, as summarized in Table 54.5. A possible explanation could be that these bacteria do not demonstrate mucus degradation properties, which is likely what occurs secondary to bacterial overgrowth in pouchitis. The role of probiotics in pouchitis in the future is likely to be as an adjunct to conventional therapy as this is an aggressive condition. Examples of probiotics used in the treatment of pouchitis are summarized in Table 54.5.

54.4.1.4 Lactose Intolerance. This is a common enzyme deficiency disorder resulting from a congenital deficiency of β-galactosidase, which helps digest lactose. It presents with symptoms such as diarrhoea, abdominal cramps, and bloating. Lactase-positive bacterial strains such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* added to milk products help to digest lactose (Marteau and others 1990). This is perhaps secondary to a reduction of lactose in the dairy product through fermentation and the release of lactase in the gastrointestinal tract by the probiotics.

54.4.2 Effects of Probiotics in Extraintestinal Disorders

54.4.2.1 Allergies. Atopic eczema due to milk allergy in infants was shown to improve following administration of probiotics. The proposed mechanism is that probiotics promote the endogenous barrier mechanism and reduce intestinal inflammation (Majama and Isolauri 1997). Other studies have shown significant differences in the

TABLE 54.5 Summary of Human Trials in Pouchitis.

Probiotic Used	No. of Subjects	Study Type	Results	Reference
VSL#3	40	Randomized controlled trial	Maintenance of remission in chronic pouchitis after antibiotic induced remission. 15% relapse rate compared with 100% in control group	Gionchetti and others 2000
VSL#3	40	Randomized controlled trial	Prevention of acute pouchitis in patients after ileo-anal pouch surgery. 10% pouchitis rate in probiotic group compared with 40% in control group	Gionchetti and others 2003
Lactobacillus rhamnosus GG	20	Randomized controlled trial	No differences were observed between the groups with regard to the mean pouchitis disease activity index	Kuisma and others 2003
VSL#3 (high dose)	36	Randomized controlled trial	Maintenance of remission in recurrent or refractory pouchitis after antibiotic induced remission. 85% remained in remission at one year, compared with 6% in placebo group	Mimura and others 2004

ratio of *bifidobacterium* to *clostridium* species in children who later develop atopy. A randomized double-blind placebo-controlled study demonstrated how perinatal administration of *L. rhamnosus strain GG* halved the incidence of atopic eczema in at-risk children during the first two years of life and this preventive effect extends to the age of four years (Kalliomaki and others 2003). Hence probiotics could have a role in the maturation of human immunity to a nonatopic state. Early priming of the immune system by commensal flora, resulting in a Th1 response rather than the more atopic Th2 response, could confer protection against allergy (Bjorksten and others 2001; Kalliomaki and others 2001).

54.4.2.2 Old Age. Elie Metchnikoff first hypothesized that a high concentration of lactobacilli in intestinal flora were important for longevity in humans (Metchnikoff 1907). In health the adult gut microflora stays stable in species composition within individuals over time (Zoetendal and others 1998). In old age there are changes in both species composition and amount. The elderly have lower amounts of *bifidobacteria* and higher amounts of yeasts, *enterobacteria*, *clostridia*, *lactobacilli*, *streptococci*, and *enterobacteria*. Restoring the gut microflora with probiotics may help restore gastrointestinal health in the elderly. Probiotics have been shown to increase transit times in human feeding studies, thereby improving nutrient absorption (Marteau and Buotron-Ruault 2002). As mentioned previously probiotics may interfere with the *in vivo* growth of *H. pylori*. This gastrointestinal infection is a common cause of morbidity in the elderly. They also showed promise in the treatment of *C. difficile* and antibiotic associated diarrhoea in clinical trials.

Immunosenescence, causing reduction in T cell population, occurs in the elderly. Phagocytes and natural killer cells are also reduced leading to increased susceptibility to infections. Immunomodulation by probiotics may help counter this effect. A clinical trial showed how probiotics were associated with a reduction in duration of illness caused by "winter" infections (Turchet and others 2003).

54.4.2.3 Hepatic Encephalopathy. In this disorder, the liver fails to break down the ammonia, among other products, produced by urease activity of intestinal bacteria so that it reaches a toxic level causing mental impairment. Lactic acid bacteria used as an add-on to treatment has shown a decrease in faecal urease activity and thus shows promise for future use (Scevola and others 1989).

54.4.2.4 Sucrase-Isomaltase Deficiency. This is not an uncommon primary disaccharidase deficiency where sucrose malabsorption occurs leading to hydrogen-producing bacterial fermentation causing bloating, cramps, and diarrhea. Symptoms disappear on sucrose-free diet. In one study *Saccharomyces cerevisiae* was used with some success to treat this condition. The authors postulate that the probiotic organism supplies the missing enzyme (Harms and others 1987).

54.4.2.5 Hypercholesterolaemia. Several lactic acid bacteria have been shown to produce bile salt hydrolase, which deconjugates bile salts in the enterohepatic circulation. This effect may reduce cholesterol (De Smet and others 1998). This mechanism of action could be similar to that of bile binding resins (e.g., cholestyramine) which bind bile acids so that the liver has to use cholesterol to produce more bile acids. It was also shown that deconjugated bile salts do not function as well as conjugated forms, which would result in less absorption of cholesterol (Reynier and others 1981). *In vitro*, lactic acid bacilli *Streptococcus HJS-1*, *Lactobacillus HJL-37*, and *Bifidobacterium HJB-4* isolated from the human intestinal tract had the ability to reduce cholesterol. One group suggests that *Lactobacillus acidophilus* reduces blood cholesterol by direct breakdown of cholesterol and deconjugation of bile salts (Gilliland and others 1985).

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Herbs in the Management of Diabetes Mellitus with an Emphasis on Ginseng

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55.1 Problem of Diabetes Mellitus 176
55.2 Opportunity for Herbs in Diabetes 177
55.3 Evidence for the Efficacy of Herbs in Diabetes 178
55.4 Evidence for the Efficacy of Ginseng in Diabetes 179
55.4.1 Ginseng and Its Components 179
55.4.2 Antidiabetic Efficacy of Ginseng and Its Components in Animals 179
55.4.2.1 Antihyperglycemic Effects of Ginseng in Animals 179
55.4.2.2 Antihyperglycemic Effects of Ginsenoside in Animals 181
55.4.2.3 Antihyperglycemic Effects of Panaxans, Quinquefolans, Eleutherans, and Ginsenans in Animals 181
55.4.3 Antidiabetic Mechanisms of Ginseng and Its Components 181
55.4.3.1 Modulation of Glucose Absorption 181
55.4.3.2 Modulation of Insulin Secretion and Binding 182
55.4.3.3 Modulation of Glucose Transport 182
55.4.3.4 Modulation of Glucose Disposal 183
55.4.4 Effect of Ginseng and Its Components on Features of the Metabolic Syndrome in Animals and In Vitro 184

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

55.4.5 Antidiabetic Efficacy of Ginseng and Its Components in Humans 185
55.4.5.1 Antihyperglycemic Effects of American Ginseng in Humans 185
55.4.5.2 Antihyperglycemic Effects of Korean Red Ginseng in Humans 188
55.4.6 Effect of Ginseng on Features of the Metabolic Syndrome in Humans 188
55.5 Limitations of the Evidence for Herbs and Diabetes 189
55.5.1 Variability in the Composition of Herbs 189
55.5.2 Variability in the Efficacy of Herbs 191
55.6 Conclusions 192
References 193

Herbal remedies and diabetes mellitus have a long shared history. Both were first described in historical accounts of several ancient cultures over 2000 years. Symptoms of diabetes that included polyuria and polydypsia were described in the Egyptian Ebers papyri, Greek Epidemics Book III of Hippocrates, the Chinese Nei Ching, and Ayurvedic writings (Cheng 2000; Sanders 2001). The treatments for these disorders were described largely as being plant based herbal remedies (Cheng 2000; Sanders 2001). Ethnopharmacological investigations have since implicated thousands of plant derived herbs in the treatment of diabetes mellitus. A comprehensive review of data for 1000 of these herbs reported that >80% have demonstrated some antidiabetic activity (Oubre and others 1997). There is, nevertheless, only one example of an approved antidiabetic drug that was developed from a herb with a long history of use for diabetes: the biguanide Metformin from French lilac (*Galega officinalis*) (Marles and Farnsworth 1995). Numerous other herbs remain candidates for antidiabetic drug development. This chapter addresses the evidence supporting the use of herbs in the management of diabetes. It starts with a discussion of the problem of diabetes, the opportunity that exists for herbs in the management of diabetes, and the state of the evidence for their clinical antidiabetic efficacy. It then proceeds to a discussion of the evidence for one of the best studied herbs, ginseng, with an emphasis on data from in vitro, animal and human studies. Finally, the limitations of the evidence supporting the clinical antidiabetic efficacy of herbs are discussed using ginseng as an example to illustrate the challenges in achieving reproducible clinical efficacy.

55.1 PROBLEM OF DIABETES MELLITUS

Diabetes mellitus is comprised of a heterogeneous group of metabolic diseases, characterized by hyperglycemia. There are two main types: type 1 and type 2 diabetes. The former is caused by the complete absence of endogenous insulin production owing to the autoimmune destruction of pancreatic β-cells. The latter is caused a combination of insulin resistance and an insulin secretory defect. Type 2 diabetes is more prevalent than type 1 diabetes, representing 90% of the cases (American Diabetes Association 2006). In >80% of people, type 2 diabetes also clusters with obesity, dyslipidemia (low HDL-cholesterol and high triglycerides), elevated blood pressure, and impaired fibrinolysis (Haffner and others 2000) This constellation of features defines the dysbolism of insulin resistance and is

termed the metabolic syndrome (Alberti and others 1998; Adult Treatment Panel III 2001; Grundy and others 2005). Because of this clustering of risk factors and the increased CVD burden that accompanies it, diabetes is now treated as a risk equivalent to established CVD in therapeutic guidelines for the Word Health Organization (Alberti and others 1998), American Heart Association (Grundy and others 2005) and National Cholesterol Education Program (Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001). Guidelines for the pharmacological and nutritional medical therapy of diabetes aggressively target the features of the metabolic syndrome, in addition to the insulin resistance and/or insulin secretory defect underlying the hyperglycemia that characterize diabetes (Alberti and others 1998; Wolever and others 2000; Grundy and others 2005; Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001).

Despite the numerous preventative strategies and armories of medication, the management of type 2 diabetes remains grossly unsatisfactory. Diabetes is emerging as a pandemic. From 1995 to 2025 the prevalence of diabetes in adults is predicted to increase by 27% in developed countries and 48% in developing countries (Gu and others 1999). In the US, the trends may already be outpacing the predictions. Data from the Centers for Disease Control show that from 1991 to 2001 diabetes increased by 61% and obesity, one of its most important precipitating factors, increased by 74% (Mokdad and others 2003). The progression of the disease has been no better attenuated. Men with diabetes have benefited far less from declines in CHD mortality, while women with diabetes have experienced an increase in risk compared with their nondiabetic counterparts (Gu and others 1999). Glycemic control targets for monotherapy also continue to go unmet in those with the disease (Turner and others 1999). With the reliance on multiple patented pharmacological agents to attain glycemic control targets, the cost of treatment has also become a real concern. Even with the use of generic drugs, the escalation of costs with multiple-therapy treatment is high. There is a 30-fold cost differential among approved diabetes treatments (Leiter and others 2002). The World Health Organization (1993) acknowledges that the ability of developing countries to afford this level of treatment is dubious. These concerns point to the need for cheaper and more effective prevention and treatment modalities.

55.2 OPPORTUNITY FOR HERBS IN DIABETES

Complementary and alternative medicine (CAM) approaches that include herbs may hold promise in diabetes. A compelling argument has been made that the random in vitro "high-throughput" screening for new drug therapies preferred by pharmaceutical companies has less practical merit than an ethnopharmacological approach that involves ethnobotany and screening of traditional systems of medicine for candidate therapies (Marles and Farnsworth 1995). This is especially true for diseases such as diabetes that are complex metabolic disorders, as certain metabolic targets of these approaches may be unrelated or secondary to effects on more proximal defects (Marles and Farnsworth 1995). The implication is that herbal treatments not identified by conventional in vitro screening systems might still be proven to have clinical efficacy and potential for development.

The public in their actions already endorse the use of herbs. Driven by the insufficiency of treatment and anecdotal evidence, paraherbalism and pseudoscience, the use of CAM

increased by 68% from 1990 to 1997 in the US (Eisenberg and others 1998). The most recent estimates suggest that 75% of general medical patients in the U.S. are now using CAM therapies (Winslow and others 2002). One of the strongest independent determinants of this behavior is the use of CAM to treat diabetes (Wolsko and others 2002). Among Canadians with diabetes, it has been reported that 37% use CAM (Ryan and others 2001). This increasingly high demand has occurred in the absence of safety and efficacy evidence, adequate regulatory standards, patient disclosures to physicians, and physician education. This has prompted a unified call from the medical community in the form of editorials, letters, and commentaries for randomized controlled clinical trials (RCTs) to evaluate CAM treatments and provide a basis for legitimate health claims (Goldbeck-Wood and others 1996; Levin and others 1997; Angell and Kassirer 1998; Hershfield 1998; Hoey 1998; Dalen 1998; Oppel 1998; Anonymous 2000; Ernst 2000).

55.3 EVIDENCE FOR THE EFFICACY OF HERBS IN DIABETES

The call for more rigorous clinical assessments of CAMs is being answered by a segment of the literature. There is a growing database of clinical trials investigating the effects of several herbs in diabetes. The efficacy, safety, and mechanisms of these herbs in diabetes have been well described in a recent systematic review of 42 randomized and 16 non-randomized clinical trials (Yeh and others 2003). The herbs with supporting clinical data in diabetes were shown to include ginseng (*Panax* spp.), ivy gourd (*Coccinia Indica*), garlic (*Allium sativum* and *Allium cepa*), holy basil (*Ocimum sanctum*), fenugreek (*Trigonella foenum graecum*), prickly pear cactus or nopal (*Opuntia streptacantha*), milk thistle (*Silibum marianum*), fig leaf (*Ficus carica*), gurmar (*Gymnema sylvestre*), bitter melon (*Momordica charantia*), Aloe vera, Gingko biloba, and various herb combinations in Traditional Chinese Medicine, Native American medicine, and Tibetan medicine. Various hypoglycemic mechanisms have been suggested for these herbs from animal and in vitro models. These include delayed glucose absorption in the gut (Aloe vera, prickly pear cactus), increased glucose uptake/disposal (fig leaf, ivy gourd), glucose stimulated insulin secretion (garlic, holy basil, gurmar), the first two (fenugreek), the last two (bitter melon), or all three (ginseng) (Yeh and others 2003; Vuksan and others 2001a, b).

The antihyperglycemic efficacy remains inconclusive for the majority of these herbs. Although determined to be safe, there is insufficient evidence to make conclusions about their efficacy (Yeh and others 2003). This is despite the direction of the evidence for a positive effect being strong. Greater than 75% of studies show improved indices of glycemic control (Yeh and others 2003). The main limitation is that individual herbs have only a small number of clinical trials, the majority of which suffer from poor quality owing to under-powering or small sample size, lack of randomization, absence of blinding, and inadequate reporting of dropouts. But strong evidence is mounting to support the use of selected herbs in diabetes. Ivy gourd and American ginseng (*Panax quinquefolius* L.) were determined to have the best evidence from adequately designed RCTs to support clinical efficacy in diabetes (Yeh and others 2003). This conclusion, as it relates to ginseng, is further supported by the position of American Diabetes Association in their evidence based nutrition recommendations (Franz and others 2002).

55.4 EVIDENCE FOR THE EFFICACY OF GINSENG IN DIABETES

The state of the evidence for ginseng serves as an example of the quantity and quality of evidence that is available to support antidiabetes claims for herbs. A detailed discussion of the in vitro, animal, and human evidence for the antidiabetic efficacy of ginseng and its components follows.

55.4.1 Ginseng and Its Components

Ginseng is an herb derived from several species of the plant family Araliaceae and genus *Panax*. It is usually consumed as dried powder in capsules, but is also available as a gel, tea, or tincture (World Health Organization 1999). Thirteen distinct species of ginseng have been identified with numerous different cultivars (British Columbia Ministry of Agriculture 1999) (Fig. 55.1). The two most popular species of ginseng are American and Asian ginsengs. Other commercial species include Korean red (steamed *Panax ginseng* C.A. Meyer), Japanese (*Panax japonicus* C.A. Meyer), Sanchi, Vietnamese (*Panax vietnamensis*), and the nonpanax species Siberian ginseng. American ginseng is indigenous to Ontario, Quebec, BC, and Wisconsin, while the other species are indigenous to Asia (World Health Organization 1999). All are genetically distinct (Mihalov 2000).

Various components of ginseng have been shown to have pharmacological activity. These include ginsenosides (dammarane-, oleanane-, and octillilol-type triterpene glycoside saponins) (NG and Yeung 1985; Attele and others 1999), ginsenans (polysaccharide), panaxans, quinquefolans, and eleuthernas (peptidoglycans) (Konno and others 1984; 1985; NG and Yeung 1985; Hikino and others 1986; Oshima and others 1987; Tomoda and others 1993, 1994), and peptide fractions (Kajiwara and others 1995). Most pharmacological action of ginseng is attributed to ginsenosides. The most common ginsenosides are the dammarane-type triterpene glycosides. These share similarities with the classical steroid hormones (Fig. 55.1).

55.4.2 Antidiabetic Efficacy of Ginseng and Its Components in Animals

55.4.2.1 Antihyperglycemic Effects of Ginseng in Animals. A substantial database of animal studies has reported improvements in longterm glycemic control with feeding of various ginseng sources. Aqueous extracts of Asian ginseng root body and rootlets each administered orally at a dose of 500 mg/kg/d body weight for 28d decreased fasting blood glucose by ~40% and ~37% with a concomitant decrease in insulin of ~76% and 52% in KKAy diabetic mice (Cheung and others 2001). Both results were comparable to reductions (37% for glucose and 67% for insulin) observed for the thiazolidinedione, rosiglitazone, administered at a dose of 0.33 mg/kg (Cheung and others 2001). Asian ginseng fed at 1000 mg/kg/d for 15–16 d reduced fasting glycemia by ~35% in streptozotocin-induced diabetic rats (Xie and others 1993). Asian ginseng root extract at 150 mg/kg significantly improved glucose tolerance by ~10% in ob/ob mice (Dey and others 2003). Asian ginseng berry extract at 150 mg/kg significantly improved glucose tolerance by ~30% in both ob/ob mice (Dey and others 2003) and db/db mice (Xie and others 2002a) after 12 d. It also improved fasting insulin and insulin sensitivity in ob/ob mice after 12 d (Attele and others 2002). Finally, 150 mg/kg American ginseng berry (Xie and others 2002b) and 50 and 150 mg/kg American ginseng leaf (Xie and others 2004) extracts were

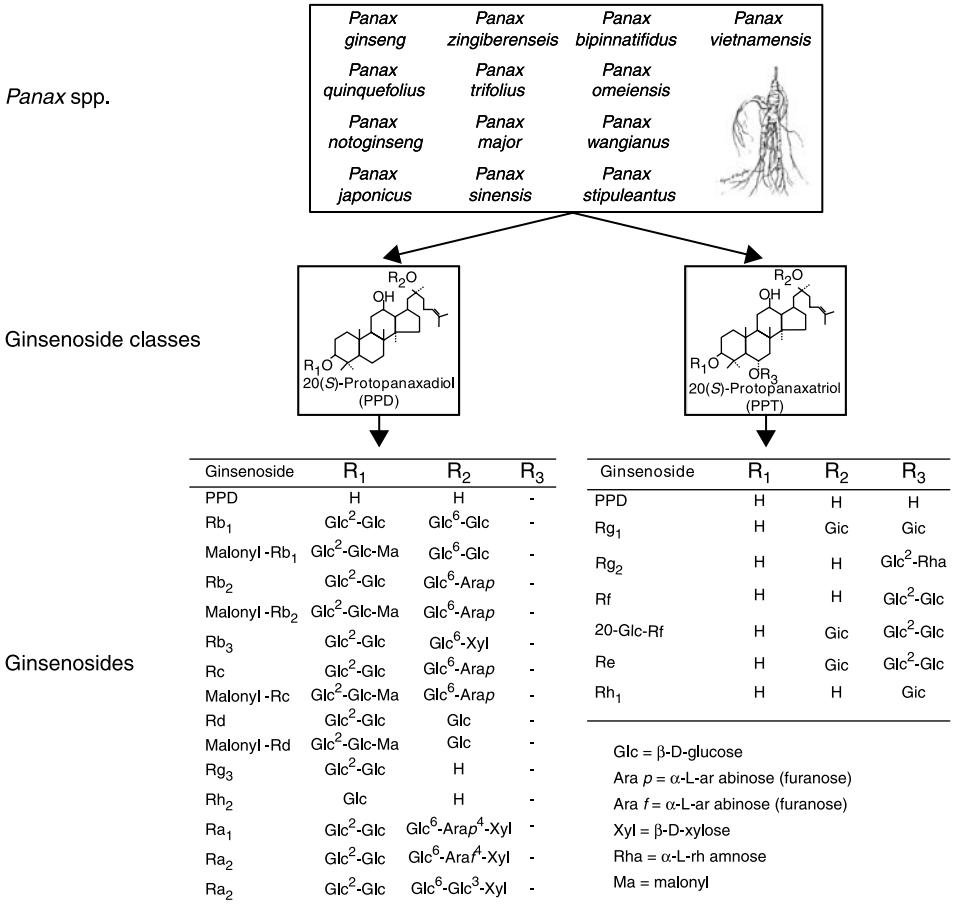

Figure 55.1 *Schematic depicting the 13 known species of ginseng and the structures of the two dammarane-type triterpene glycoside classes of ginsenosides derived from them with examples of the most common individual ginsenosides derived from each class. The individual ginsenosides and the composition of their glycosidic side chains are tabulated below each structure. Adapted from British Columbia Ministry of Agriculture (1999), NG and Yeung (1985), and Attele and others (1999).*

able to replicate the improvements in glucose, insulin, and body-weight control in ob/ob mice after 12 d.

Acute reductions in glycemia have also been reported following various ginseng sources. A water extract of Asian ginseng injected intravenously at 100 mg/kg with glucose decreased glycemia significantly compared with glucose alone in diabetic mice (Kimuru and Suzuki 1991). An oral injection of a water extract of Asian ginseng decreased glycemia at doses of 200 and 400 mg/kg by ~18 and 28% in normal mice and by ~23 and 36% in epinephrine induced hyperglycemic mice (Ohnishi and others 1996). Methanol extracts of Chinese, Korean red, Ontario grown American, and Sanchi ginsengs administered by stomach intubation decreased glycemia in resting mice from ~5–15% compared with placebo (Martinez and Staba 1984). And established extracts of Asian ginseng administered intraperintoneally reduced resting glycemia, epinephrine induced hyperglycemia,

and intravenous glucose tolerance: *DPG-1* at 360 mg/kg (Kimura and others 1981), *DPG-3-2* from 3 to 100 mg/kg (Morita and others 1985), *EPG-3-2* at 50 mg/kg (Kimura and others 1981; Hitonobu and others 1982), and *Fraction 4* at 10 mg (Yokozawa and others 1975). Taken together, these data suggest that ginseng possesses acute hypoglycemic effects.

55.4.2.2 Antihyperglycemic Effects of Ginsenoside in Animals. Specific ginsenosides may play a role in the effects observed with whole ginseng and its extracts. Various ginsenosides and their classes have been shown to decrease glycemia directly in various animal models. For example, the most prevalent PPT ginsenoside, Rg_1, was shown to decrease glycemia in resting mice by 17% compared with placebo when administered by stomach intubation at 50 mg/kg (Martinez and Staba 1984). Rb_2, a PPD ginsenoside, was also noticed to decrease glycemia in streptozotocin induced diabetic rats after 6 d at 10 mg/kg by intraperintoneal injection (Yokozawa and others 1985a). An Asian ginseng extract high in Re significantly reduced glycemia in alloxan diabetic mice (Kimura and others 1981). Finally, Re decreased fasting glycemia in ob/ob mice after 12 d at 20 mg/kg by intraperintoneal injection (Attele and others 2002).

55.4.2.3 Antihyperglycemic Effects of Panaxans, Quinquefolans, Eleutherans, and Ginsenans in Animals. The role of non-ginsenoside components in the antihyperglycemic effects of ginseng cannot be precluded, although their effects have only been observed following intraperintoneal injection. Panaxans A-U from Asian ginseng (Konno and others 1984; Konno and others 1985; Ng and Yeung 1985), quinquefolans A-C from American ginseng (Oshima and others 1987), and eleutherans A-G from Siberian ginseng (Hikino and others 1986) have all shown marked yet differential hypoglycemic effects when administered as intraperintoneal injections at doses from 10 to 300 mg/kg in both normal and alloxan induced hyperglycemic mice. No studies investigating the effects of ginsenans on glycemia could be found in the literature.

55.4.3 Antidiabetic Mechanisms of Ginseng and Its Components

How ginseng through its various fractions has its effect on glycemic regulation is not clear. In vitro and animal data support four possibilities: modulation of (A) glucose absorption, (B) insulin secretion, (C) glucose transport, and/or (D) glucose disposal (Fig. 55.2). A discussion of each follows.

55.4.3.1 Modulation of Glucose Absorption. There is indirect in vitro and animal evidence to suggest that ginseng may affect the rate of digestion. An inhibition of neuronal discharge frequency from the gastric compartment of the brain stem in rats by American ginseng has been observed (Yuan and others 1998). Inhibition of gastric secretion by Chinese ginseng has also been observed in rats (Suzuki and others 1991). The result of both may be to slow the digestion of food, decreasing the rate of carbohydrate absorption into portal hepatic circulation. This suggestion is supported by more direct evidence. More recent data showed that the addition of Asian ginseng inhibited both glucose and maltose stimulated duodenal muscle movement, as assessed by short circuit current in isolated rat and human duodenal mucosa (Onomura and others 1999).

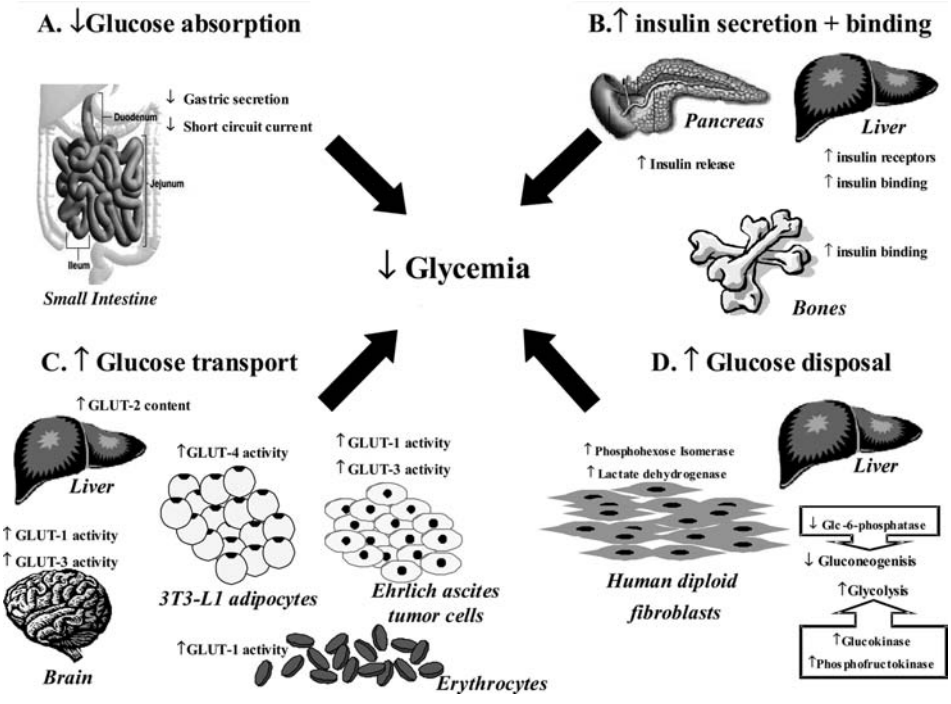

Figure 55.2 *Schematic depicting the four possible antihyperglycemic mechanisms of action of ginseng and its components: (a) modulation of glucose absorption, (b) modulation of insulin secretion and binding, (c) modulation of glucose transport, and (d) modulation of glucose disposal. The site of action and specific observations at that site are shown for each mechanism. Please refer to the accompanying text for greater detail and references. GLUT denotes glucose transporter.*

55.4.3.2 Modulation of Insulin Secretion and Binding. Both in vitro and animal data indicate that various ginseng extracts and ginsenosides increase insulin secretion and binding. DPG-3-2 stimulated insulin biosynthesis in mice islets and rat pancreas at concentrations from 0.5 to 1.0 mg/ml (Kimura and others 1981b; Hitonobu and others 1982). EPG-3-2 increased glucose stimulated insulin secretion in alloxan diabetic mice at doses from 10 to 50 mg/kg after intraperintoneal injection (Waki and others 1982). Total ginsenosides from Asian ginseng increased glucose and nonglucose stimulated insulin release from rat islets in a dose dependent manner at doses from 0.10 to 0.25 mg/ml (Li and Lu 1987). Rg_1 increased insulin binding with a ~2-fold increase in the total number of binding sites in rat liver and brain 3 d and 5 d after three consecutive days of intraperintoneal injections at 10 mg/kg/d (Tchilian and others 1991). An Asian ginseng extract with 40% total ginsenosides/saponins increased insulin binding by ~4-fold in the bone marrow of older rats at an intra-gastric dose of 100 mg/kg/d for 12 d (Yushu and Yuzhen 1988). Finally, panaxan B increased glucose stimulated insulin secretion in a dose-dependent manner at doses from 0 to 10 mg/kg with a maximal increase that was 6-fold higher than control and increased insulin binding 5 h after intraperintoneal injection in normal mice (Suzuki and Hikino 1989).

55.4.3.3 Modulation of Glucose Transport. A growing database of in vitro studies suggests that ginseng and some of its components may increase glucose transport in

various cell lines. A water extract of Chinese ginseng increased GLUT-2 protein in the livers of normal and hyperglycemic mice (Ohnishi and others 1996). PPT, (20R)-PPD, Rg_1, Rc, Rd, Re, Rf, Rg_2, Rh_1, Rb_1, and Rb_2 increased 2-deoxy-D-[2-^{3}H]-glucose uptake in isolated sheep erythrocytes by GLUT-1 in a dose dependent manner at doses from 0.01 to 10 μM (Hasegawa and others 1994). The standardized Asian ginseng extract G115®, which has a high ratio of PPD:PPT (Cui 1995), increased 2-deoxy-D-[2-^{3}H]-glucose uptake in a dose dependent manner in rabbit brain at doses from 23 to 46 μg/ml (Samira and others 1985) and Ehrlich ascites tumour cells at doses from 0.5 to 10 μg/ml (Yamasaki and others 1993). Presumably these increases in uptake were mediated principally by GLUT-1 and/or GLUT-3, as neither tissue expresses SGLT-1, GLUT-2, or GLUT-4 (Au and others 1997) and the GLUT-5 in brain has only low affinity for glucose (Sheppard and Kahn 1999). A water extract of Asian ginseng increased basal (non-insulin stimulated) 2-deoxy-D-[2-^{3}H]-glucose uptake in 3T3-L1 adipocytes at a dose of 100 μg/ml, presumably through GLUT-4 (Hong and others 2000). Finally, panaxan B increased glucose disappearance during an insulin suppression test at an intraperintoneal dose of 10 mg/kg after 5 h in normal mice (Yushu and Yuzhen 1988).

These direct effects on glucose transport are further supported by primary effects of ginseng on related intracellular signaling systems. An effect on the peroxisome proliferator activated receptor-γ (PPAR-γ), the target of the thiazolidinediones, has been observed. The aqueous extract of Asian ginseng rootlets increased PPAR-γ protein comparably with rosiglitazone in KKAy mice (Chung and others 2001). Ginsenosides Rh1 and Rh2 also decreased PKC activity at concentrations from 10 to 100 (M, via a concomitant decrease in its allosteric activator diaclyglycerol (DAG), in NIH 3T3 fibroblasts (Byun and others 1997). Other effects included decreasing effects of ginsenosides Rb1, Rb2, and Rc on tumor necrosis fator-α (TNF-α) in human macrophages at concentrations from 10 to 100 M (Cho and others 2001). Similar changes have been related to improvements in insulin sensitivity (Itani and others 2002; Ruan and others 2002).

55.4.3.4 Modulation of Glucose Disposal. Data from various animal models of diabetes suggest that different ginseng extracts and ginsenosides may increase glucose disposal. The saponin fraction from G115® at a dose of 200 μg/ml in human diploid fibroblasts increased the glycolytic enzyme, phosphohexose isomerase, and several isozymes of the pyruvate lactate shunt enzyme, lactate dehydrogenase, although no changes in intracellular or extracellular glucose concentrations or glucose uptake accompanied these changes (Shia and others 1982). Oral administration of aqueous extracts of Asian ginseng root body and rootlets at a dose of 500 mg/kg for 28 d decreased the activity of the rate limiting gluconeogenic enzyme glucose-6-phophatase (G6Pase) in liver preparations of KKAy diabetic mice by 46% and 20% (Chung and others 2001). Asian ginseng berry extract at 150 mg/kg and American ginseng leaf at 50 and 150 mg/kg significantly increased glucose disappearance in ob/ob mice after 12 d (Attele and others 2002; Xie and others 2004). Finally, Rb_2 increased the activity of the rate limiting glycolytic enzymes phosphofructokinase and pyruvate kinase, while decreasing the activity of the rate limiting gluconeogenic enzyme G6Pase in liver from normal rats after a single 10 mg intraperintoneal injection (Yokazawa and others 1984b) and increased the activity of glucokinase while decreasing the activity of G6Pase in liver from streptozotocin diabetic rats after intraperintoneal injection at 10 mg/d for 6 d (Yokozawa and others 1985a). These mechanisms are also supported by the same primary cellular signaling mechanisms described above for improvements in glucose transport.

55.4.4 Effect of Ginseng and Its Components on Features of the Metabolic Syndrome in Animals and In Vitro

Ginseng and its components been shown to have effects on several features of the metabolic syndrome in animal and in vitro models. First, an extract of Asian ginseng berries with high Re improved obesity, inducing weight loss in db/db (Xie and others 2002a) and ob/ob mice (Attele and others 2002).

Second, Korean red ginseng and its total ginsenosides (Yamamoto and others 1983a; Inoue and others 1999), fractions 3 and 4 (Yamamoto and others 1983b, c), a lipophillic ginseng fraction (Park and others 1996), Rb_1 (Park and others 2002), and Rb_2 (Yokazawa and others 1984a, 1985b) improved the dyslipidemia that characterizes the syndrome, decreasing serum triglycerides while increasing serum HDL-cholesterol in the rat. This is in addition to related effects that included decreasing serum cholesterol (Sakakibara and others 1975; Ikehara and others 1978; Yamamoto and others 1983b, c; Yokazawa and others 1984a, 1985b), plasma free fatty acids (Yamamoto and others 1983c), and liver total cholesterol (Sakakibara and others 1975; Ikehara and others 1978; Park and others 2002) and increasing clearance of ^{14}C-labeled cholesterol from plasma and excretion of C^{14}-labeled cholesterol and its metabolites (bile acids, saponifiable material, sterols) from the liver (Yamamoto and others 1983c).

Third, different ginseng species and their fractions improved hypertension. Sanchi ginseng decreased both systolic and diastolic blood pressure (Lei and Chiou 1986) and total ginsenosides from Asian ginseng decreased mean blood pressure in a dose dependent manner in the rat (Kang and others 1995a). An aqueous Asian ginseng extract (Toda and others 2001), Korean red ginseng preparations with increasing concentrations of Rg_3 (Kim and others 2001), total ginsenosides (Kang and others 1995b), PPTs, Rg_1, Rg_3 (Kim and others 1999a, b), Re (Kang and others 1995a; Scott and others 2001), and Rb_1 (Scott and others 2001) also produced related increases in endothelium-dependent relaxation mediated by nitric oxide and cGMP (Kim and others 1983b; Kang and others 1995a, b; Kim and others 2000; Scott and others 2001; Toda and others 2001) and endothelium-independent relaxation mediated by potassium channels (Kim and others 1983a) in studies in animal aorta, cerebral arteries, and ventricular myocytes.

Fourth, various ginseng species and their fractions improved impaired fibrinolysis. Japanese ginseng (*Panax japonicus* C.A. Meyer) (Matsuda and others 1989), notoginsenoside-R1 (Zang and others 1997), Rg_1 (Ushio 1992; Matsuda and others 1986b), Rg_2, Rg_3, Re, Rb_1, Rb_2, Rc (Matsuda and others 1986a), and Ro (Matsuda and others 1986a, b) increased fibrinolysis mediated by increases in tissue plasminogen activator (tPA) (Ushio 1992; Zhang and others 1997) and urokinase (Matsuda and others 1986b, 1989) and decreases in plasminogen activator inhibitor-1 (PAI-1) (Zhang and others 1997). An Asian ginseng extract (Cui and others 1999), Korean red ginseng (Yun and others 2001), the lipophilic fraction of Asian ginseng (Park and others 1996, 2002), Rb_1, Rb_2, Rc, Re, Rg_1, Rg_2, Ro (Matsuda and others 1986b), and Rg_3 (Jung and others 1998) have also produced related inhibitory effects on platelet aggregation and adhesion in rabbit, rat, and human platelets (Matsuda and others 1986b; Park and others 1995; Jung and others 1998; Cui and others 1999) and coagulation, assessed by increased activated partial prothrombin time (APPT), prothrombin time (PT), and thrombin time (TT) in platelet-poor rat plasma (Yun and others 1987; Park and others 1996; Jung and others 1998).

Taken together, various ginseng sources and ginsenosides have been shown to improve features of the metabolic syndrome in animal models that include obesity, dyslipidemia, hypertension, and impaired fibrinolysis. These effects are in addition to the observed

antihyperglycemic effects of ginseng and its components and can be considered complementary in reducing diabetes and cardiovascular disease risk in animal models.

55.4.5 Antidiabetic Efficacy of Ginseng and Its Components in Humans

Until recently, there was very limited data in humans to support the traditional use of ginseng in diabetes and confirm the hypoglycemic effect of ginseng observed in animal and in vitro models. Only a small group of flawed published studies were accessible. Sotaniemi and coworkers (1995) reported that 8 weeks of treatment with 100 and 200 mg/d of an unspecified ginseng improved fasting glycemia and longterm glycemic control, assessed by HbA1c, respectively in 36 type 2 diabetic subjects. But the results were ambiguous due to significant weight loss differences between the treatment groups and poorly described statistics. In another study, Tetsutani and coworkers (2000) reported that 24 months of treatment with a Korean red ginseng extract at doses from 3 to 4.5 g decreased HbA1c in 34 people with type 2 diabetes compared with controls. But the subject selection, allocation to treatment, statistics, and follow-up of the study were very poorly described. Secondary sources also reported glycemic benefits of Korean red ginseng in diabetes (Anonymous 2005). But control groups were not reported and primary sources could not be retrieved for verification.

55.4.5.1 Antihyperglycemic Effects of American Ginseng in Humans. The need for good human data prompted us to initiate a clinical testing program to explore the acute and chronic effects of American ginseng in humans. We conducted a series of five randomized placebo controlled acute clinical studies (Table 55.1) to evaluate the efficacy of American ginseng (*Chai-Na-Ta Corp.*, BC) in lowering postprandial glycemia and its dosing and timing effects in subjects with and without diabetes using a 25 g-OGTT protocol. The principal goal was to identify an efficacious dosing and timing schedule for of a single batch of American ginseng for a longterm study. The main findings were 4-fold: (1) American ginseng reduced postprandial glycemia from 9.1 to 38.5%; (2) doses from 1 to 9 g were equally efficacious; (3) times from 0 to 120 min before the glucose challenge were equally efficacious in diabetic subjects without interaction with their usual antiihyperglycemic therapy; and (4) only AG >40 min before the OGTT reduced glycemia in nondiabetic subjects. We concluded that the selected batch of American ginseng at a dose of 1 g, administered as an oral agent 40-min before each meal was sufficient to reduce acute postprandial glycemia (Vuksan and others 2000a–d, 2001c).

Although this acute clinical testing program identified an efficacious treatment protocol, it was unclear which part of American ginseng's profile gave rise to the acute antihyperglycemic effects. To address this issue, we measured the ginsenoside composition of the American ginseng used in all of the above acute studies (Table 55.2). It was noticed that it had a high proportion of PPD (Rb_1, Rb_2, Rc, and Rd) relative to PPT (Rg_1, Re, Rf) ginsenosides, a ratio smaller than one for the ratios Rg_1/Re and Rb_2/Rc, and Rf was absent. All of these features of its composition indicated that ginseng was of the genus and species selected (Ma and others 1996; Wang and others 1999; Awang 2000). Although it was tempting to suggest that these features might be responsible for its effects, without a basis for comparison we concluded that they were interpretable only for authentication (Vuksan and others 2000a–d, 2001c). Other unmeasured components could have played an independent or interactive role. These include >25 different ginsenosides, >10 peptidoglycans (quinquefolans for American ginseng), various ginsenans, numerous peptides and fatty acids, and countless other organic compounds. It was

TABLE 55.1 Summary of Five Acute Clinical Studies Assessing the Dosing and Timing Effects of a Single American Ginseng (AG) Source on Postprandial Glycemia.

Study	Sample	OGTT Dose	AG Dosing	AG Timing	% AUC Reductions	*P*-value
Vuksan and others (2000a)	10 NGT (Age: 34 ± 2 years, BMI: 25.6 ± 1 kg/m^2)	25 g	3 g	−40 min	18% ↓ for 3 g AG @ −40 min vs. placebo	$P < 0.05$
			3 g	0 min	No effect for 3 g AG @ 0 min vs. placebo	$P =$ NS
Vuksan and others (2000a)	9 DM2 (Age: 62 ± 2 years, BMI: 29 ± 1.7 kg/m^2, HbA_{1c}: 7.6 ± 0.2%)	25 g	3 g	−40 min	22% ↓ for 3 g AG @ −40 min vs. placebo	$P < 0.05$
			3 g	0 min	19% ↓ for 3 g AG @ 0 min vs. placebo	P < 0.05
Vuksan and others (2000c)	10 NGT (Age: 41 ± 4 years, BMI: 24.8 ± 1.1 kg/m^2)	25 g	3, 6, 9 g	−120, −80, −40 min	26.6, 29.3, 38.5% ↓ for 3, 6, and 9 g vs. placebo	$P < 0.05$
					No effect of timing	$P =$ NS
Vuksan and others (2000d)	10 DM2 (Age: 63 ± 2 years, BMI: 27.7 ± 1.5 kg/m^2, HbA_1c: 7.3 ± 0.3%)	25 g	3, 6, 9 g	−120, −80, −40, 0 min	19.7, 15.3, 15.9% ↓ for 3, 6, and 9 g vs. placebo	$P < 0.05$
					No effect of timing	P = NS
Vuksan and others (2001c)	12NGT (Age: 42 ± 7 years, BMI: 24.1 ± 1.1 kg/m^2)	25 g	1, 2, 3 g	−40, −20, −10, 0 min	14.4, 10.6, 9.1% ↓ for 1,2, and 3 g vs. placebo	$P < 0.05$
					14.1, 15.0, 9.2% ↓ for −40 min vs. −20, −10, and 0 min	$P < 0.05$

Source: *NGT, BMI, DM2, OGTT, and AUC denote normal glucose tolerance, body mass index, type 2 diabetes mellitus, oral glucose tolerance test, and area under the curve respectively. P-values are for comparisons between absolute values using repeated measures ANOVA adjusted with the Newman–Keuls procedure. Data are mean ± SEM.*

TABLE 55.2 Energy, Nutrient, and Ginsenoside Profile of the Placebo and American Ginseng Source used in Five Acute Clinical Trials.

	Content	
Constituent	Placebo	American Ginseng
Energy (Kcal)	3.51	3.44
Macronutrients		
Carbohydrate (g)	0.73	0.57
Fat (g)	0.039	0.013
Protein (g)	0.069	0.26
Ginsenosides		
(20S)-Protopanaxadiols (PPD) (%w/w)		
Rb_1	–	1.53
Rb_2	–	0.06
Rc	–	0.24
Rd	–	0.44
PPD subtotal	–	2.27
(20S)-Protopanaxatriols (PPT) (% w/w)		
Rg_1	–	0.1
Re	–	0.83
Rf	–	0
PPT subtotal	–	0.93
Total (% w/w)	–	3.21
Ratios (% w/w: % w/w)		
PPD : PPT	–	2.44
$Rb_1 : Rg_1$	–	15.3
$Rb_1 : Rc$	–	0.25
$Rg_1 : Re$	–	0.12

Adapted with permission from Vuksan and others (2001a).

concluded that replication of the findings with an American ginseng designed to have a similar profile was the most viable way to offer evidence for the efficacy of this ginsenoside profile.

The ginsenoside profile and treatment protocol identified in our acute clinical testing program were applied to a longterm study. We designed an American ginseng extract (*Chai-Na-Ta Corp.*, Langley, BC) to have a ginsenoside profile similar to that of the American ginseng used in the 5 acute studies (Table 55.2; Vuksan and others 2000a–d, 2001c): total ginsenosides of 3.54 and a PPD:PPT ratio of 2.4. An 8-week double-blind, placebo-controlled crossover trial was then undertaken in which the designed American ginseng extract or placebo at a dose of 1 g was administered as an oral agent 40 min before each meal (3 g/day) in 24 subjects with type 2 diabetes (Vuksan and others 2000e). The primary outcomes were markers of glycemic control. Fasting glucose and HbA_{1c} were decreased on the American ginseng extract compared with placebo after 8 weeks. There was also an observable but insignificant increase in insulin suggesting a possible improvement in β-cell function. These benefits occurred without increasing adverse events or altering hepatic, renal, haemostatic, or blood pressure function. Taken together, the data represented proof of two concepts. First, standardization of key features of the ginsenoside profile may lead to reproducible effects. But whether the standardized ginsenoside component is driving this reproducibility is unclear. It may only be acting only as a proxy for other non-measured ginsenosides or nonsaponin components such as the peptidoglycan fraction (quinquefolans). Second, our acute postprandial testing model used to select the most efficacious ginsenoside profile, dose, and time of

administration successfully predicted longterm safety and efficacy of a source of American ginseng in type 2 diabetes. The ~15–20% glycemic lowering efficacy seen acutely (Vuksan and others 2000a–d, 2001c) was sustained in this longterm investigation.

55.4.5.2 Antihyperglycemic Effects of Korean Red Ginseng in Humans. To test whether the batch, preparation, dosing, and timing of another species of ginseng could be selected to have longterm efficacy using the same acute postprandial testing program, a similar approach was applied to Korean red ginseng (steam treated *Panax ginseng* C.A. Meyer) (Sievenpiper and others 2002). We conducted a preparation-finding study of different Korean red ginseng root fractions followed by a dose-finding study of the most efficacious fraction. Double-blind, randomized, within-subject designs were used in both studies. In the batch-finding study, seven healthy subjects received 6 g placebo and Korean red ginseng preparations of rootlets, root-body, and whole root H_2O extract 40 min before a 50 g-OGTT. In the dose-finding study, 12 healthy subjects received 0 g (placebo), 2 g, 4 g, and 6 g of the most efficacious root fraction following the same protocol. The studies were successful in identifying efficacious preparation and dose of Korean red ginseng. In the preparation-finding study, a wide variation in the ginsenoside profiles was achieved across the three root fractions. This variation coincided with differential effects, although the PPD:PPT ratio was unrelated. Rootlets decreased AUC by 29% compared with placebo, while neither the H_2O extract nor root-body affected glycemia. In the dose-finding study, the rootlets were tested as the most efficacious fraction. A significant effect of rootlets treatment (mean of three doses) but not dose was found. The mean of three doses decreased AUC by 17% compared with placebo. Taken together the studies indicated that rootlets given at a dose of 2 g, 40-min before a meal was sufficient to achieve reproducible reductions in postprandial glycemia. This Korean red ginseng treatment protocol was indicated for longterm study (Sievenpiper and others 2006).

The most efficacious preparation (Korean red ginseng rootlets), dose (2 g), and mode of administration (oral preprandial agent at −40-min) selected from the sequential acute preparation- and dose-finding studies were subjected to longterm testing. A double-blind, randomized, placebo-controlled, crossover trial was conducted. Nineteen type 2 diabetic subjects received 2 g placebo or Korean red ginseng rootlets 40 min before each meal (6 g/d) for 12 weeks, while maintained on their conventional diabetes treatment. Fasting plasma insulin and 75 g-OGTT derived AUC plasma insulin were significantly decreased on the selected Korean red ginseng treatment compared with placebo. This occurred while fasting plasma glucose was unchanged and 75 g-OGTT derived AUC plasma glucose was significantly decreased. The combination was reflected in an identical 33% increase in both the homeostasis model assessment (HOMA) and the 75 g-OGTT derived insulin sensitivity indices on the selected Korean red ginseng treatment compared with placebo. These benefits occurred without increasing adverse events or altering hepatic, renal, haemostatic, or blood pressure function. We concluded that our acute testing program identified a Korean red ginseng preparation, dose, and mode of administration that improved longterm glucose and insulin regulation safely beyond conventional treatment in type 2 diabetes (Vuksan and others 2006).

55.4.6 Effect of Ginseng on Features of the Metabolic Syndrome in Humans

Additional effects of various ginseng sources on features of metabolic syndrome have been observed in humans. First, weight-controlling effects of ginseng have been reported.

In the clinical diabetes trial of Sotaniemi and coworkers (1995), 100 and 200 mg/d of the unspecified ginseng improved obesity, decreasing weight after 8 weeks of treatment compared with baseline.

Second, lipid and lipoproteinemia improving effects have been observed. Korean red ginseng at a dose of 4.5 g/d (1.5 g before each meal TID) improved dyslipidemia, decreasing triglycerides and increasing HDL cholesterol after 7 days in an uncontrolled pilot study of five normal and six hyperlipidemic men (Yamamoto and others 1983). In our longterm randomized, double-blind, crossover study with the American ginseng extract, total-cholesterol, LDL-cholesterol, and the total-/HDL-cholesterol ratio were reduced compared with placebo, with an observable but insignificant increase in HDL cholesterol in subjects with type 2 diabetes (Vuksan and others 2001d).

Third, antihypertensive effects have been observed in different cohorts. Different ginseng preparations improved various indices of blood pressure function. Korean red ginseng at a dose of 4.5 g/d (1.5 g before each meal TID) decreased 24 h mean systolic blood pressure compared with placebo after 8 weeks in 26 subjects with essential hypertension in a nonrandomized, unblinded, crossover study with a shortened placebo phase (4 vs. 8 weeks) (Han and others 1998). The Asian ginseng (*Panax ginseng* C.A. Meyer) extract *Ginsana G115* (Pharmaton, Ridgefield, CT, USA) significantly decreased acute blood pressure 2 h after ingestion compared with baseline (Caron and others 2002). Korean red ginseng significantly increased forearm blood flow responses, consistent with mediation by nitric oxide, in seven hypertensive test subjects compared with 10 untreated hypertensive control subjects (Sung and others 2000). In our longterm study with the American ginseng extract, both systolic and diastolic blood pressures were significantly reduced compared with placebo in subjects with type 2 diabetes (Stavro and others 2000). But two follow-up studies from our clinic with various American ginseng preparations showed null effects in hypertensive subjects (Stavro and others 2005, 2006).

Finally, improvements in haemostatic parameters have been observed with ginseng in subjects with type 2 diabetes. In our longterm study with the American ginseng extract, a significant reduction in plasminogen activator inhibitor-1 (PAI-1) was observed from baseline. But the comparison with placebo was only approaching significance (Vuksan and others 2001e).

Taken together, various sources of ginseng, especially Korean red and American ginseng, have been observed to improve different metabolic disorders related to diabetes in various human cohorts. In addition to impaired glycemia, these include prominent features of the metabolic syndrome: obesity, dyslipidemia, hypertension, and impaired fibrinoloysis. As these additional metabolic effects have been observed across different ginseng preparations, doses, disease models, and investigator groups, they offer robust support for the efficacy of American and Korean ginseng sources in reducing diabetes and cardiovascular disease risk in humans.

55.5 LIMITATIONS OF THE EVIDENCE FOR HERBS AND DIABETES

55.5.1 Variability in the Composition of Herbs

The evidence for the antidiabetic efficacy of herbs comes with serious limitations. Our experience with ginseng serves as an example. To quantify the extent of the variability in the active components found in ginseng, we undertook a meta-analysis of the coefficient-of-variation (CV) in ginsenosides across ginseng type (batch, preparation, variety, species), assay-technique, and ginsenoside-type. Thirty-two articles met the inclusion criteria. Together these articles reported ginsenoside concentrations for 317

batches of ginseng. Ginseng-type comprised 10 levels of *Panax* species, their preparations, and their varieties: 121 Asian (cultivated) (*Panax ginseng* C.A. Meyer (cultivated)), 36 Asian (red) (*Panax ginseng* C.A. Meyer (red)), three Asian (wild) (*Panax ginseng* C.A. Meyer (wild)), 35 Asian (extract) (*Panax ginseng* C.A. Meyer (extract)), 74 American (cultivated) (*Panax quinquefolius* L. (cultivated)), four American (wild) (*Panax quinquefolius* L. (wild)), seven American (extract) (*Panax quinquefolius* L. (extract)), 12 Japanese (*Panax japonicus* C.A. Meyer), 10 Pseudo (*Panax pseudoginseng* WALL.), and 10 Sanchi (*Panax notoginseng* (Burk.) F.H. Chen) ginseng batches. Assay technique comprised six levels of different assay techniques: 213 HPLC-UV, 43 GC-MS, 33 HPLC-MS, 12 DCC, nine HPLC-differential refractometry [DR], and four HPLC-ELSD. Ginsenoside-type comprised 21 levels of ginsenoside indices: the protopanaxadiol (PPD) ginsenosides (207 Rb_1, 197 Rb_2, 199 Rc, 202 Rd, 34 Rg_3), protopanaxatriol (PPT) ginsenosides (202 Rg_1, 230 Rf, 208 Re, 105 Rg_2) their sums (228 PPD, 229 PPT, 252 Total) and ratios (239 PPD:PPT, 255 Rb_1:Rg_1, 197 Rb_2:Rc, 261 Re:Rb_1, 262 Rc:Rb_1, 258 Rd:Rb_1, 259 Rb_2:Rb_1, 254 Rf:Rb_1, 207 Rg_1:Re). This meta-analysis demonstrated a high CV in ginsenosides across the three main factors: 26–103% for ginseng-type, 31 to 81% for assay-technique, and 36 to 112% for ginsenoside-type (Fig. 55.3) with the differences in ginseng-type dependent on the assay-technique used. This analysis demonstrated that the ginsenoside composition of ginseng is highly variable across different ginseng source parameters (Sievenpiper and others 2004a).

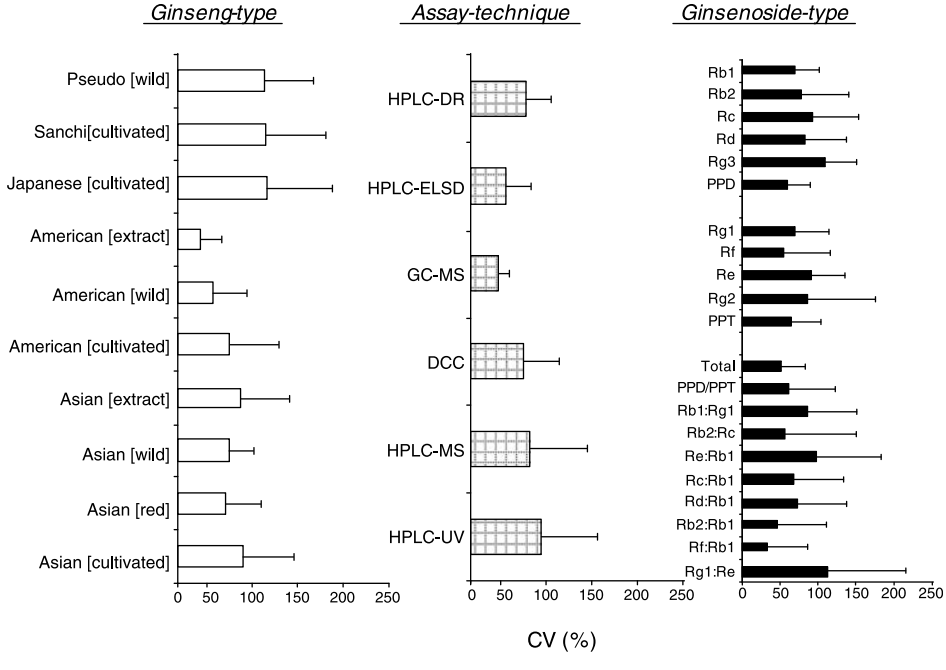

Figure 55.3 *Coefficient-of-variation (CV) of ginsenoside concentrations for the main effects species, assay-technique, and ginsenoside-type. CV of ginsenoside concentrations were calculated as CV = SD/mean x 100% in a three-factor block design. A blocking principle was applied to the data such that each level of each factor (10 levels of species, six levels of assay-technique, and two levels of ginsenoside-type) was crossed with each level of the other factors for the calculation of CV. The CV data calculated for each possible combination were pooled and meaned for each level of each factor. As a result, CV data are mean ± SD. Adapted from Sievenpiper and others (2004a).*

55.5.2 Variability in the Efficacy of Herbs

There is evidence that this high variability in composition may contribute to equally high variability in efficacy. Variable pharmacological effects appear secondary to differences in composition. We conducted a series of acute, blinded, placebo-controlled clinical studies, to assess the effect of increasing ginsenoside variability across similar ginseng source parameters of progressively greater ginsenoside variability (batch, preparation, variety, and species) on postprandial glycemia. A 75 g-oral-glucose-tolerance-test (75 g-OGTT) protocol was followed with ginseng administered 40 min before the start of each test. The ginsenoside variability we were able to achieve experimentally across the ginseng source parameters was equal to the actual variability seen across similar parameters in the meta-analysis. This coincided with highly variable glycemic effects. In the first study, while our original efficacious batch of American ginseng again demonstrated acute postprandial glycemic lowering efficacy, a second batch with a depressed ginsenoside profile including a low PPD:PPT ratio was ineffective (Sievenpiper and others 2003a). In the next two studies, another species, Asian ginseng, with marked inversions in its ginsenoside profile (PPD:PPT < 1) had null and opposing effects on plasma glucose indices (Sievenpiper and others 2003b). Finally, in the most recent study, in which eight of the most common ginseng types with distinct ginsenoside profiles were compared head-to-head, decreasing, null, and increasing effects was observed. A third batch of American ginseng lowered plasma glucose, while Japanese-rhizome (*Panax japonicus* C.A. Meyer), Sanchi (*Panax notoginseng*), Vietnamese-wild (*Panax vietnamensis*), and Korean red ginsengs had null effects and Asian (Sievenpiper and others 2004b), Siberian (*Eleutherococcus senticosus*), and American-wild ginsengs significantly raised acute postprandial plasma glucose (Fig. 55.4). The PPD:PPT ratio was implicated as the sole independent predictor of four of seven plasma glucose and insulin outcomes in this study (Sievenpiper and others 2004b). But the variance explained by this ginsenoside ratio was $<7\%$, bringing into question its utility as a marker for standardization. Again other unmeasured saponin or nonsaponin components could have played independent or interactive roles. Taken together, the high variability in acute postprandial glycemia appeared secondary to the variability in the ginseng source (batch, preparaprtion, variety, and species) and its composition, as represented by the measured ginsenoside profile, specifically the PPD:PPT ratio.

The implications of this high variability is that the evidence for efficacy and safety reported for specific herbal products may not be generalizable to other over-the-counter species, varieties, preparations, and batches of the herb. Although this concern makes a compelling argument for better regulatory standards, there are mitigating factors. One limitation is that no basis for standardization exists. For example, it is not clear which of the >30 ginsenosides and their ratios should be standardized in ginseng. The independent and interactive roles of other principles such as panaxans (peptidoglycans) and ginsenans (polysaccharides) are also unknown. The implication is that even with standardized products, uncertainty remains. Another important limitation is the assay. No universal assay exists for most compositional factors. The observed interaction between ginseng-type and assay-type seen in the meta-analysis of the CV in ginsenoside concentrations may produce biases in comparisons among ginseng samples. The same set of concerns is applicable to all herbal treatements.

Taken together, these limitations indicate the need to tie reference components to efficacy and establish specific assay criteria. Positive steps in this direction are being taken. In the case of ginseng, we reviewed above a growing database of rigorously conducted animal and in vitro studies that are pointing to different ginsenosides for

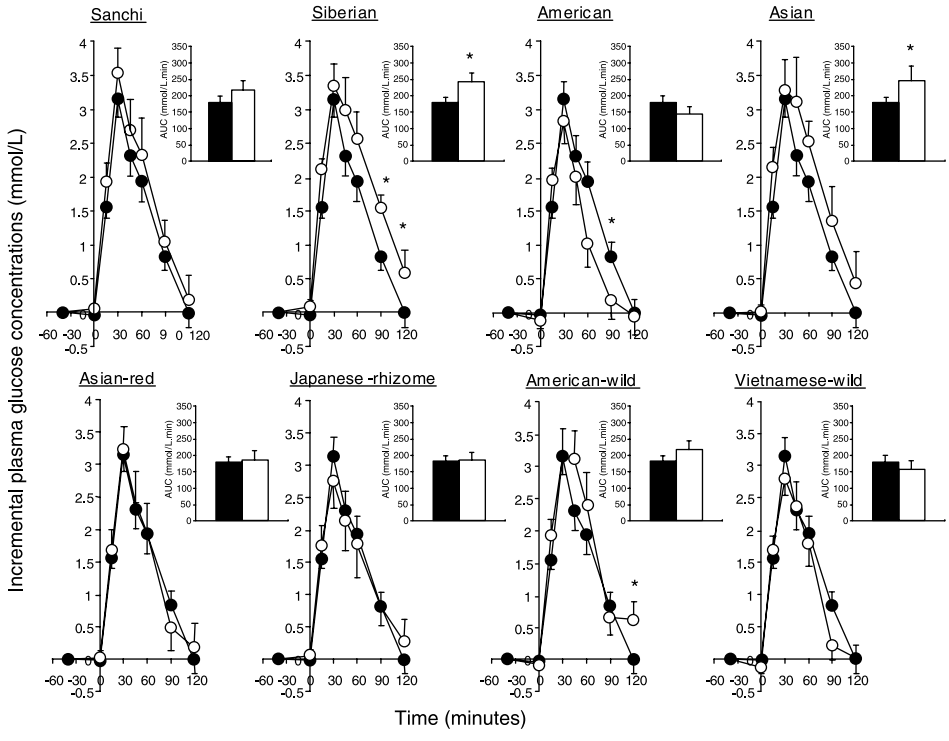

Figure 55.4 *Decreasing, null, and increasing effects of eight common types of ginseng on postprandial plasma glucose. The line plots and bars in the array represent the incremental change and area under the curve (AUC) for the mean of 2 identical placebos (•) or one of eight of the most popular ginseng types, Sanchi, Siberian, American, Asian, Asian-red, Japanese, American-wild, or Vietnamese-wild ginseng, (o) administered at a dose of 3 g 40 min before a 75 g-OGTT in 12 nondiabetic subjects (sex: 6 m:6 f, age: 34 ± 3y, BMI: 25.8 ± 1.2 kg/m^2). Asterisks indicate that points or bars for ginseng are significantly different from placebo ($P < 0.05$, one-way repeated measures ANOVA with non-orthogonal contrasts). Data are mean ± SEM. Adapted from Sievenpiper and others (2004b).*

antihyperglycemic indications. Some of the most promising isolated components, for which there is consistent data across different models, species, doses, and investigator groups include Re (Spinas and others 1998; Attele and others 2002), Rb2 (Yokozawa and others 1984b, 1985a; Hasegawa and others 1994), and panaxan B (Yushu and Yuzhen 1988; Suzuki and Hikino 1989). The American Botanical council (2006) has also initiated the largest ginseng evaluation program with the development of a common HPLC-UV assay. Finally, our clinic is presently undertaking a stepwise assessment of the effect of progressively smaller ginseng fractions on acute postprandial glycemia in nondiabetic subjects.

55.6 CONCLUSIONS

In conclusion, although the evidence from traditional systems of medicine through to randomized controlled clinical studies is largely aligned in supporting a diabetes indication for selected herbs, such as Ivy gourd and American and Korean red ginsengs, the reproducibility of their safety and efficacy remains questionable. The extensive evidence reviewed for ginseng illustrates this point. There is a large database of animal and in vitro studies that support the effect of various ginseng sources and ginsenosides and non-ginsenosides

components (panaxans, quinquefolans, eleutherans, and ginsenans) on glycemic regulation, effector mechanisms, and features of the metabolic syndrome. Added to this scientific foundation is a growing database of clinical studies that has twice shown that a ginseng source can be selected using an acute postprandial screening model to have long-term efficacy and safety in people with type 2 diabetes. Despite these advances, further clinical investigations have revealed compelling caveats. Not all ginseng sources exhibit the same magnitude or direction of effects in humans. Highly variable acute glycemic effects have been observed secondary to the ginsenoside profile, as it varies across ginseng batch, preparation, variety, and species. Variability across other unmeasured saponin and nonsaponin compositional factors also cannot be ruled out. The clinical consequences of this compositional variability may be that its efficacy and safety in diabetes will be equally highly variable. This situation necessitates that compositional markers of its antihyperglycemic effects be identified. Without these data the consumer cannot be assured of the safety and efficacy of ginseng products and the call from the medical community for randomized controlled trials and standardization of ginseng are moot. Although some leads have emerged, it remains unclear which species, varieties, batches, and preparations of ginseng have antihyperglycemic efficacy and which saponin or nonsaponin components confer this efficacy. The implication is that a basis for standardization for ginseng or other less well studied herbs is premature. One alternative may be to conduct batch-to-batch efficacy screening to identify efficacious batches using the same stepwise acute postprandial clinical screening model we have developed. But this alternative seems practically and financially untenable on an industrial scale. Future research directed at the identification of active components becomes the only viable means of supporting efficacy claims for herbs. In the absence of such standardization, consumers should remain cautiously optimistic about the use of herbs in diabetes.

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56

Functional Foods and Minerals: Calcium

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56.1 Introduction 201
56.2 Definitions 202
56.3 Macrominerals: Calcium 203
56.3.1 Calcium and Cancer 203
56.3.1.1 Colon Cancer 203
56.3.1.2 Prostate Cancer 205
56.3.1.3 Other Cancers 206
56.3.1.4 Summary 206
56.3.2 Calcium and Weight Control or Weight Loss 207
56.3.3 Calcium and Osteoporosis 207
56.3.4 Calcium and Blood Pressure 210
56.4 Summary of the Relationships Between Calcium-Rich Foods and Reduction of Disease 210
56.5 Implications for the Consumption of Calcium-Rich Functional Foods 211
References 212

56.1 INTRODUCTION

The designation of "functional food" applies to foods that have a naturally occurring bioactive ingredient, that is, nutrient or phytochemical molecule, with major benefits to health beyond basic nutrition. Each bioactive ingredient exists in sufficient amounts in one serving of a functional food to either promote health, prevent disease, or both.

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

These functional foods almost invariably carry a health claim if they are processed and packaged. Many examples of functional foods exist, but this chapter focuses on dairy products that provide calcium, or calcium plus vitamin D. Calcium-rich functional foods may have beneficial effects in decreasing the risks of a few tissue-specific cancers, they may also contribute to weight loss and to reduction of blood pressure, and they may lower the risk of skeletal fractures. Most human studies showing health benefits of calcium from dairy foods or from foods plus supplements of calcium have been population-based or epidemiologic, but others have been experimentally or clinically based. The highest value placed on any type of study is now accepted to be prospective randomized clinical trials (RCTs) because they have well defined end-points and they essentially link cause (etiologic risk factors) and effect. The major strength of RCTs is the randomization of subjects in human experiments to a treatment or control group. Even though other types of human studies, especially observational ones, may help investigators by generating hypotheses for the design of RCTs, they have lower scientific value because they do not meet these two criteria. This conservative approach to basing clinical decisions on the findings of RCTs is now commonly accepted as the scientific basis for medical therapy. Furthermore, meta-analyses of RCTs on the same diet-disease linkage are quite powerful in influencing medical decision-making for prevention or treatment of specific diseases.

The health effects of other macronutrients, especially magnesium and potassium that are found in rich supply in plant foods, have not been investigated to any considerable degree as calcium has. So, little information is available for review of research on these minerals at this time. The emphasis on calcium in this review, therefore, reflects the extensive investigation of this single mineral, either by itself or with vitamin D, on several health or disease endpoints.

56.2 DEFINITIONS

Functional foods, in the context of the definition of the term, include one or more ingredients that have a positive impact on bone mass or density, and that may help reduce skeletal fractures. New lines of investigation have been attempting to pinpoint benefits of calcium on the risk reduction of cancers and the loss of body weight resulting from adequate intakes of calcium on long-term basis. Only a few foods providing sufficient calcium fit this rather stringent definition. These are dairy foods, that is, milks, cheeses, yogurts, and related derivative products of milk. Soy milks, while important sources of calcium consumed by strict vegetarians and others, are not reviewed here because so little research has examined the roles of calcium from soy milk in health promotion and disease prevention. For the same reason, calcium-fortified foods per se are not reviewed.

Calcium is a mineral on the Generally Recognized as Safe (GRAS) list of the Food and Drug Administration that has multiple functions as a fortificant, in addition to its role as a basic nutrient. Because the upper limit (UL) of safety for calcium has long been established as 2500 mg/day, calcium fortification of suitable food vehicles has been generally considered safe. Concerns arise, however, regarding the potential, especially in conjunction with high intakes of vitamin D, of soft tissue calcifications, including those of arterial walls, and renal stones.

This review focuses on the health effects of calcium derived from foods obtained from cows' milk, from fortified foods, and calcium provided by daily supplements (pills).

Although other mammalian species provide milks rich in calcium, relatively few human studies have examined the calcium-health/disease linkages of milks other than those from cows.

56.3 MACROMINERALS: CALCIUM

The major macromineral with a rich literature on health/disease relationships is calcium, and so review of recent reports of calcium and its potential benefits on cancer, weight control, osteoporosis, and hypertension is offered. In several published reports calcium has been administered to experimental subjects along with vitamin D. So, unraveling the effects of one or another or both nutrients has not been easy; further comment on the results of research investigations involving both calcium and vitamin D are provided in this review.

The most positive data relating calcium intake to risk reduction of cancer applies to colon cancer for which a number of reports have found declines in colon or colorectal cancer when consumption patterns were adequate in calcium or when calcium supplements were taken. (By far, most of the studies have investigated colon cancer; only a few have examined rectal cancer, so that this review will cover only colon cancer.) Less positive support exists for an adverse role of calcium on prostate cancer risk reduction. The effects of calcium on bone health and osteoporosis prevention are weakly positive, and, therefore, less robust than often portrayed. The linkage of calcium to hypertension is weak, despite numerous reports attempting to show a major reduction of blood pressure following lengthy periods of calcium supplementation. The highlights of these reports are covered next.

56.3.1 Calcium and Cancer

Reports on this broad topic of calcium and cancer are numerous; so emphasis is on reports that have been published in the last few years. Most of the literature cited has focused on colon cancer, and much less on prostate and other cancers. Often calcium intake is closely related to vitamin D intake, but these two nutrients may have differing effects relating to cancer development or its prevention. For example, calcium and vitamin D may both may be considered protective against colon cancer, but they may have opposite effects regarding prostate cancer. Nutrients in dairy foods other than calcium that may also exert anticancer roles, including conjugated linoleic acids, but they are not reviewed herein.

56.3.1.1 Colon Cancer. The first major research report on the postulated linkage between vitamin D/calcium and lower risk of colon cancer was published in 1980 (Garland and Garland 1980). Several case-control studies have also supported the beneficial effects of calcium on colon cancer, but, because prospective trials provide more acceptable study methodology, they serve as the basis of the review of the calcium-colon cancer relationship.

A meta-analysis on this linkage published in 1996 concluded that calcium did not protect against colorectal cancer, but more recent prospective investigations have essentially reversed this earlier meta-analysis (Bergsma-Kadijk and others 1996). Why has this switch in perspective occurred? An accumulation of new evidence from more carefully designed studies has resulted in better understandings of the mechanisms through

which calcium ions may be influencing the carcinogenic process in colonic epithelial cells. Vitamin D has also been speculated to have a role in the benefit accrued from calcium, but too little research has been conducted on this vitamin alone for review here.

Colonic cancer development is a multiple-step process that begins with the formation of adenomatous polyps as the initial part of the adenoma–carcinoma sequence. Adenomas (polyps) may grow in size and develop increasing levels of disorganization or dysplasia. A small proportion of adenomas develop into carcinomas (cancer). Several genes are involved in the different stages, but the local colonic environment, including intraluminal nutrients, modifies cellular functions either favorably or unfavorably. Many of the clinically based investigations have studied patients with recurrent adenomas in order to demonstrate benefits of calcium, typically calcium supplements which increase total calcium intake to within the range of 1200–2000 mg per day, in reducing new polyp formation. These investigations, published in the last several years, have strongly supported a protective effect of calcium in reducing the risk of colon cancer. A few of these reports are noted.

Baron and others (1999) demonstrated that calcium supplements (1200 mg per day) given subjects with an increased risk for cancer because of a history of prior adenomas in treated subjects versus control subjects given a placebo. This was the first carefully designed randomized clinical trial on this relationship, and it essentially overruled the earlier studies that found no effect of calcium, including the meta-analysis (Bergsma-Kadijk and others 1996). Two other published reports of randomized clinical trials by Hofstad and others (1998) and Bonithon-Kopp and others (2000) arrived at a similar conclusion, namely calcium supplementation over several years significantly reduced colonic adenomas. Grau and others (2003) who used a supplement of both calcium and vitamin D found a significant reduction in adenomas and, based on their results, they suggested that sufficient dietary calcium was necessary for a beneficial effect of vitamin D.

A U.S. Cancer Prevention Study (not a trial) generated results from dietary intakes obtained from over 120,000 men and women that support a modest lowering of the risk of colorectal cancer with higher calcium intakes (those in the upper quintiles). The investigators reported a benefit of vitamin D only for men (McCullough and others 2003).

Peters and others (2004a) reported on almost 3700 patients with histologically verified adenomas of the distal colon and compared them to almost 35,000 subjects with no adenomas. Calcium intakes from foods and supplements were assessed in all subjects and then the subjects were split into quintiles according to total calcium intake. Colonic adenoma risk declined by 27% in subjects who consumed 1200 mg of calcium or more per day than for control subjects (low- or non-users of supplements) whose total calcium intake was less than $\sim$1000 mg. The significant decline in risk of adenomas was attributed by the authors to their supplement use that raised their total calcium intake to greater than 1200 mg a day (Peters and others 2004a).

The Women's Health Initiative (Wactawski-Wende and others 2006) found no reduction in colorectal cancer in postmenopausal women administered supplements of calcium plus vitamin D compared to women given placebo. This study has been criticized because the dose of vitamin D, 400 IU per day, is now considered too low to be effective. Other aspects of this study have also come under attack. Further investigations are needed, while addressing these deficiencies, to determine if calcium plus vitamin D, at a dosage of 800–1000 IU per day, is effective in reducing colon cancer. Both an adequate intake of calcium, 1000 mg per or so, and of vitamin D are now considered important for reducing the risk of colon cancer (Fleet 2006). Dietary or biosynthesized vitamin D may be critical

for mucosal cell maintenance of the active D metabolite, calcitriol, in colonic tissue, according to speculation based on results of a study of mice (Cross and others 2006).

Two major mechanisms of action of calcium in reducing colon cancer risk have been put forth, and they are not exclusive of each other. The first is that calcium ions, not absorbed by the small bowel, are able to bind to organic acids, such as free fatty acids and bile acids, within the lumen, thereby removing these potentially carcinogenic acids for elimination in stools. The other major mechanism has been newly proposed: calcium ions act with a calcium-sensing receptor (CaSR) found on the luminal membrane surface of epithelial cells of the colonic mucosa (Lamprecht and Lipkin 2003), and thereby modify intracellular signaling that is associated with the risk of advanced adenomas (Peters and others 2004a,b). It is presumed that both mechanisms may operate simultaneously to reduce the carcinogenic effects of bile acids on the colonic cells and also to improve intracellular calcium ion pathways that reduce cell proliferation but promote cell differentiation, depending on stage of cell differentiation (Buras and others 1995). Intracellular calcium may also help to regulate apoptosis in healthy cells. Finally, new understandings from animal studies suggest that colonic cells themselves help to maintain concentrations of the vitamin D hormone, calcitriol (also known as 1,25-dihydroxyvitamin D), which in turn keeps calcium ion concentrations up within these cells (Buras and others 1995). Folate may also be required in maintaining cell calcitriol levels by serving as methyl donors.

56.3.1.2 Prostate Cancer. The hypothesized positive linkage between calcium (and vitamin D) and prostate cancer remains rather tenuous after more than a decade of research. Yet, the idea that calcium ions may reduce prostate proliferation and inhibit carcinogenesis remains intriguing. Some reports, however, suggest that vitamin D may be the beneficial factor in preventing prostate cancer (Giovannucci 2005). Two reports of large prospective cohorts even concluded that high calcium intakes increased the risk for prostate cancer (Rodriguez and others 2003; Giovannucci and others 2006). A leading idea about the adverse effect of calcium on prostate cancer relates to the intracellular suppression by calcium of the metabolic conversion of vitamin D to its final hormonal form, calcitriol, which is considered beneficial for the prevention of prostate cancer (Giovannucci 2005). A recent report by Giovannucci and others (2006) issued a warning about the consumption of too much calcium by middle-aged men who may be at increased risk of prostate cancer. This cautious view is also supported by a meta-analysis (Gao and others 2005). Thus, the potentially different roles of these two dietary nutrients in reducing the risk of prostate cancer make it critically important that better understandings of their functions within prostate cells be obtained. If calcium is beneficial in colonic mucosa cells, why should it not also be beneficial, either alone or along with vitamin D, in prostate cells? Does vitamin D have a beneficial effect independent of calcium in colonic cells but not in prostate cells? Do these two nutrients regulate cell proliferation in a differential way? These and other questions regarding prostate caner etiology need answers.

The mechanisms of action of calcium ions and vitamin D, in the hormonal form, on prostate cells appear to be different than for colonic mucosal cells. Calcium ions apparently do not have effects on the CaSR on membrane surfaces of prostate cells because of the absence or low numbers of CaSR, as colonic epithelial cells do, but it remains possible that an intraluminal role of calcium ions exists for slowing proliferation and/or normalizing differentiation and apoptosis in prostate cells (Cross and others 2006). The

hormonal form of vitamin D acts on its nuclear receptors and activates vitamin D response elements of DNA, thereby promoting normal cell proliferation and differentiation, as well as supporting normal rates of apoptosis. The vitamin D hormonal receptor exists in practically all cell types of the body, not just in cells of the intestinal epithelium or bone. Understandings of the molecular biology of this receptor are still unfolding.

Observational and case-control studies have not provided such useful information that can help ferret out mechanistic understandings. No randomized clinical trials have been reported, but one recent report of an epidemiological cohort suggests that dairy sources of calcium and vitamin D may even increase the risk of prostate cancer (Tseng and others 2005). So, the postulated effects of calcium and vitamin D require further investigation using well-designed RCTs that can sort out the different effects of calcium and vitamin D on prostate cancer.

56.3.1.3 Other Cancers. Although other cancers, such as breast and ovarian cancers, may respond to dietary calcium or vitamin D in a positive way, too few investigations have been reported to generate a meaningful review. Vitamin D, without calcium, has been proposed to have potentially significant risk lowering effects for these reproductive cancers. The benefit of vitamin D, although promising, is supported by limited studies, none being a RCT.

56.3.1.4 Summary. The reports on colon cancer strongly support a beneficial effect of calcium or calcium plus vitamin D on the reduction of risk for colon cancer, but the same cannot be stated for prostate cancer at this time, since RCTs are lacking. Table 56.1 includes the findings of the more critical RCTs linking calcium intake and risk reduction of colon cancer. The mechanism of action by which calcium exerts its beneficial effects has not been fully established, but investigators suggest that, for colon cancer, unabsorbed calcium ions may combine with bile acids and other organic molecules with acidic groups within the lumen of the large bowel, thus preventing their uptake of these potentially carcinogenic acids by colonic epithelial cells. Mechanisms for a role of calcium ions in prostate cancer have not been elucidated. The concern that too high an intake of calcium may actually induce prostate cancer puts a dampener on supplementation of middle-aged men with calcium and vitamin D.

TABLE 56.1 Recent Findings of Randomized Clinical Trials Linking Supplemental Calcium Intake and the Reduction of Risk of Colon or Colorectal Cancer.

Reference	Treatment (Supp.) Daily[a]	Duration	Significant Outcome
Baron and others (1999)	Calcium (1200 mg)	12 months +48 months	Decreased recurrent adenomas (moderate)
Hofstad and others (1998)	Calcium (1600 mg)	36 months	Decreased adenoma formation
Bonnithon-Kopp and others (2000)	Calcium (2000 mg)	36 months	Modest reduction in adenomas (not signif.)
Grau and others (2003)	Calcium (1200 mg) & adequate vit. D status	48 months	Decreased recurrent adenomas

[a]Placebo used for control groups. Vit. = vitamin. Signif. = significant.

TABLE 56.2 Summary of Two Randomized Clinical Trials that Support the Relationship Between Calcium and Weight Reduction.

Reference	Treatment Daily[a]	Duration	Significant Outcome
Zemel and others (2004)	Ca supplement (500, 800, or 1200 mg)	6 months	6, 9, or 11% weight decrease; similar fat losses
Zemel and others (2005)	Dairy foods (milk or yogurt)	12 months	Weight reduction

[a]Placebo used for control groups.

56.3.2 Calcium and Weight Control or Weight Loss

Reports by researchers suggest that weight reduction in regular consumers of dairy products results from the large amounts of calcium consumed in these foods on a daily basis. This emerging area of investigation has stimulated great interest because of the current epidemic of overweight and obesity in the United States. Nevertheless, no randomized clinical trials have been performed on the calcium-weight relationship and the proposed mechanism through which calcium or vitamin D exerts its weight-lowering or appetite suppressing effect(s) have not been satisfactorily established.

In two recent publications examining women (Lin and others 2000; Zemel and others 2004), data suggest that increased intake of calcium-rich foods, either milk or yogurt, resulted in modest, though, significant weight loss over periods of 12 weeks or longer. The weight reduction in young healthy women may help prevent overweight (Lin and others 2000), but the study of weight loss in obese older women supports the benefits of dairy foods in helping these subjects lose weight (Zemel and others 2004). A recent abstract of a multicenter study which showed that, as a result of high dairy food consumption, the lean compartment of body composition may be increased along with a decline in the fat compartment (Zemel and others 2005). Other reports have generated similar conclusions.

Several possible mechanisms have been propounded to explain loss primarily in the fat compartment of the body, but evidence in support of a role for calcium or calcium plus vitamin D is limited.

In summary, these few reports support a very modest beneficial effect of calcium on the reduction of body weight. Complete data from RCTs are needed before the promise of the calcium-induced weight loss can be scientifically assessed. Table 56.2 includes a summary of the one RCT that has examined the relationship between calcium and weight reduction. In addition, the postulated mechanisms of action of calcium in contributing to weight reduction which remain elusive need further documentation.

56.3.3 Calcium and Osteoporosis

The long-held view that calcium intakes at or approximating the amounts recommended prevent or delay osteoporosis and related fractures. This generalization is only weakly supported by a scientifically more rigorous meta-analysis of randomized clinical trials that used calcium supplementation of postmenopausal women to try to improve bone density (Shea and others 2002). In the conclusion of this meta-analysis, the authors stated: “Calcium supplementation alone has a small positive effect on bone density. The data show a trend toward reduction in vertebral fractures, but do not meaningfully address the possible effect of calcium on reducing the incidence of nonvertebral fractures”

(Shea and others 2002). As developed next, skeletal gains from adequate calcium intakes are most robust in pre- and postpubertal boys and girls, especially when the calcium is provided by foods, rather than during late life. Calcium supplements of boys and girls have also been shown to increase bone mass and density, but once indistinguishable from those of control subjects within 6–12 months (Johnston and others 1992). So, adequate amounts of calcium provided by foods are more likely able to sustain optimal measurements of bone tissue than calcium supplements.

The most critical need for calcium intakes that help meet the recommended intakes of the Food and Nutrition Board of the Institute of Medicine (IOM 1997) relates to the development of peak bone mass during the prepubertal and postpubertal years of both boys and girls, that is, the span of years from approximately 8–18 years for girls and 10–20 years for boys. During this decade of early life, the accrual of bone mineral mass reaches almost 95% of total mass (sometimes referred to as optimal mass) of an individual during a lifetime (Matkovic and others 2005). Therefore, only about 5% of additional mass is gained during the decade of the 20s or slightly longer (Tylavsky and others 1989). This so-called consolidation may be enhanced by lengthy lactations of 6 months or longer by adult women, because of the large increase of "new" bone formation if calcium intake is adequate (Anderson and Rodano 1996). Bone mass typically begins to decline, though only slightly, during the latter part of the decade of the 1930s, especially in females. Although the strongest positive results of adequate calcium intakes have been shown for the growing skeleton, most of the calcium studies using supplements have involved postmenopausal women in efforts to reduce the risk of osteoporotic fractures.

A large literature exists on the relationship between calcium and bone, but only a few prospective RCTs using calcium supplementation compared to placebo have been reported for postmenopausal women. Data from studies of early postmenopausal women (less than 10 years since the menopause) suggest that calcium has practically no benefit to vertebral bone mineral density (BMD) and presumably to other skeletal sites (Dawson-Hughes and others 1990). On the other hand, later postmenopausal women (10 years or longer since the menopause) did receive some benefit, especially in modestly improved BMD of the lumbar vertebrae, but little or no benefit at other skeletal sites such as the proximal femur (hip) (Dawson-Hughes and others 1990; Elders and others 1994) (Table 56.3).

TABLE 56.3 Summary of Randomized Clinical Trials on the Relationship Between Calcium and Reduction of Osteoporotic Fractures of the Hip and Nonvertebrae or of All Skeletal Sites.

Reference	Treatment (Daily)[a]	Duration	Significant Outcome Fracture Reduction(s)
Dawson-Hughes and others (1990)	Ca supplement (1000 mg)	6 months	Vertebrae of women: >5 years postmenopause
Elders and others (1994)	Ca supplement (1000 or 2000 mg)	24 months	Vertebrae of women (perimenopausal)
Chapuy and others (1992)	Ca (1200 mg) and vitamin D (800 IU)	18 months	Hip & nonvertebral sites of women
Dawson-Hughes and others (1997)	Ca (500 mg) and vitamin D (700 IU)	36 months	Nonvertebral sites; only men significant
Larsen and others (2004)	Ca (1000 mg) and vitamin D (400 IU)	41 months	All sites of men and women

[a]Placebo used for control groups.

No studies of postmenopausal women using calcium-rich foods have been reported, but the dairy foods might have additional skeletal benefits because of the other nutrients they contain, including phosphate. The calcium: phosphate ratio of dairy foods is near 1 : 1, which provides less of a perturbation on calcium homeostasis than a supplement containing only calcium. These calcium-rich foods with phosphates may actually improve bone through short term increases in PTH that stimulate the bone formation activities of osteoblasts in bone tissue. This short-term effect of PTH has only been recognized in the last few years as contributing to bone-building. Studies of such foods in elderly subjects, especially postmenopausal women, are needed to verify the hormonal changes and increments in BMD. Vitamin D-fortified foods may possibly have an added boost in improving bone measurements. Supplements alone may not be as effective in stimulating PTH-mediated bone formation.

The proposed mechanisms for any skeletal benefit of calcium supplements in elderly women are two-fold. A slight increase in serum calcium ions resulting from intestinal absorption has an inhibitory effect on the secretion of parathyroid hormone (PTH) that works through the CaSR on cell membranes. The reduction in serum PTH in turn leads to a lower rate of skeletal resorption operating via osteoclasts and other osteolytic cells. The second mechanism, which implies an increase in intestinal calcium absorption mediated by the active hormonal form of vitamin D, that is, $1,25(OH)_2$vitamin D, provides more calcium ions for movement into bone and an increase in bone mass and BMD. Other mechanisms could also be operating to improve bone.

Another approach used by a few investigators to reduce skeletal fractures has been the combination of calcium plus vitamin D supplements. A few recent reports showing significant benefits of this combination treatment used in RCTs suggest that both calcium and vitamin D, at least in the northern latitudes of North America and Europe when vitamin D skin biosynthesis is low or zero, are needed to reduce the risks of osteoporotic fractures (Chapuy and others 1992; Dawson-Hughes and others 1997; Larsen and others 2004) (Table 56.3). When coupled with vitamin D, calcium supplements have helped to reduce significantly hip and other non-vertebral fractures (Chapuy and others 1992).

Still another approach has been the supplementation with vitamin D alone, but this preventive strategy works well only when calcium intake from foods is near recommended intake levels. Studies of vitamin D supplementation alone, though not all investigations have had significantly positive outcomes, have generally resulted in modest skeletal improvements in late postmenopausal women and older men. One vitamin D investigation in particular showed improved serum 25-hydroxyvitamin D and reduced parathyroid hormone concentrations in 50-year-old men and women administered 100 μg per day over a period of 6 months (Veith and others 2004). The investigators found this dosage level was also safe.

Because almost all of these studies have used calcium supplements at doses typically of 1000 mg per day or more, the abrupt initial perturbations in calcium regulation must be adjusted to by skeletal changes, that is, increases in bone formation and reductions in bone resorption, in order to obtain long-term gains in bone mass and density. In addition, the gains in bone mass and density following calcium supplementation, as considered in a meta-analysis, have been shown to be small or possibly nonmeasurable (Shea and others 2002). The long-term adaptations clearly lead to very modest increases in the bone measurements, as long as the supplements are continued. When they are stopped, bone measurements revert almost 100% of the time to presupplementation bone values (or worse). No comparable studies of postmenopausal women have been performed using

calcium-rich foods like milk, cheese, and yogurt. Also, only a few studies have been performed in elderly men so that conclusions cannot be made for this gender.

To sum up, although calcium supplementation results in only small gains in bone mass and density at almost every stage of the life cycle, it may be beneficial for many individuals because calcium exerts an inhibitory effect on the secretion of parathyroid hormone and, hence, on osteoclastic bone resorption. A tolerable upper limit of safety (UL) of 2400 mg exists and individuals who approach or exceed this amount from dietary sources and supplements may be at increased risks of soft-tissue calcification. So, consumption at or above the UL is of concern now in the United States because of the increasing prevalence of arterial calcification, such as of the aorta, and of potentially more serious calcifications of the kidneys and other organs.

56.3.4 Calcium and Blood Pressure

The relationship between calcium and blood pressure has been examined in many studies using adult men and women, but the findings have been controversial because so many other factors – both dietary and lifestyle – also affect blood pressure. Also, very few RCTs have been reported. The recent Dietary Approaches to Stopping Hypertension (DASH) studies which use a modified dietary approach to reduce blood pressure, including low-fat dairy foods, have shown modest lowering of both systolic and diastolic pressures in compliant subjects (Appel and others 1997; Sacks and others 2001). These two major DASH reports of RCTs have been widely cited for their beneficial effects, but the dietary changes have included, in addition to increased calcium, extra servings of fruits and vegetables that provide potassium, and little or no processed foods that typically contain sodium. So, a direct role for calcium alone in lowering blood pressure, if one exists, must be so small that it is difficult to measure in a consistent way to provide scientific validity.

56.4 SUMMARY OF THE RELATIONSHIPS BETWEEN CALCIUM-RICH FOODS AND REDUCTION OF DISEASE

The established benefits of calcium-rich functional foods on health are largely limited to bone and the reduction of osteoporotic fractures, but information about cancer, hypertension, and body weight/fat is being generated by new research investigations. The benefits of calcium from foods or supplements in RCTs have been limited to small gains (or incremental improvements in treatment groups compared to placebo or no treatment in control groups) in bone mass or density of adults over extended periods ranging from a year to several years, but the benefits are generally small, that is, 0.5–1.0%, even though these benefits are statistically significant (Shea and others 2002). Such statistically significant improvements have not been found for calcium alone (or calcium plus vitamin D) in other prospective studies, and results of too few RCTs have been reported for any solid conclusions about the calcium–disease relationships.

Potentially adverse effects of high consumption patterns of calcium or calcium plus vitamin D are of some concern in populations that are physically inactive and have high rates of adults with the metabolic syndrome. Such adult individuals are now known to be more likely to have calcified (mineralized) plaque in major arteries and potentially in kidney tissue, besides renal stones. Any site of calcification has serious effects, but

renal calcification may have deadly consequences. So, the question arises if nutritionists and other health professionals may be "pushing" too much calcium and vitamin D on the sub-population that already has adequate consumption of both calcium and vitamin D. Individuals who truly need greater amounts of these nutrients, as determined by U.S. dietary surveys such as NHANES and Canadian surveys, are generally the poor and less well educated. U.S. subpopulations include migrants and minorities, especially those with darker skin color who generate less vitamin D by skin synthesis. This issue of who benefits the most from functional foods containing calcium and possibly vitamin D needs further investigation and research support by federal agencies because the problem of low consumption of these nutrients may have broader consequences for a variety of chronic diseases than previously recognized. Nevertheless, calcium and vitamin D-enriched functional foods can be appropriately used to help prevent and to treat several diet-related chronic diseases in the developed nations of the world and help reduce deficiencies of calcium and/or vitamin D in the less developed world.

A further note of caution about calcium and vitamin D supplementation is raised by recent studies demonstrating a potential increase in arterial calcification in adults who already have significant plaque formation in their vessels rather than entry of the calcium ions into bone. How serious this shunting of calcium is for health remains uncertain now.

56.5 IMPLICATIONS FOR THE CONSUMPTION OF CALCIUM-RICH FUNCTIONAL FOODS

At this time, no FDA-approved health claims exist for (1) calcium and the reduction of risk of site-specific cancers, (2) calcium and the reduction of body weight, and (3) calcium and the reduction of blood pressure. A claim does exist, however, for calcium and the reduction of risk of osteoporosis. A logical progression would be the eventual approval of the additional claims for calcium, as reviewed herein. Yet, the scientific basis for each potential claim must be established first. Although the data for cancer, weight reduction, and hypertension are of considerable suggestive value, the cumulative research is not yet sufficiently strong to warrant approval of health claims for these. New trials and supportive findings on the calcium-rich functional foods are necessary before advanced scientific understandings of prevention of cancer or high blood pressure or weight reduction can be accepted by a consensus of the scientific community. Until new claims become established, the consumption of calcium according to the recommendations of the Food and Nutrition Board of the IOM (IOM 1997) remains the best guide for U.S. and Canadian populations. Functional foods containing both calcium and vitamin D may make the best combination of fortification because of the long-established dietary deficits of calcium and the newly recognized deficits of vitamin D among North American populations (Hanley and Davison 2005).

Several public health implications result from this review, as follows:

1. Calcium intakes that approximate the recommended intake levels for adults (IOM 1997) may have several physiologic benefits on cells and tissues that help maintain health and reduce the risks of several diseases or conditions. These benefits exist throughout life. Getting calcium from foods rather than supplements may be preferable because excessive intake may be better avoided.

2. Vitamin D intakes at recommended levels for adults (IOM 1997) are now considered to have been set too low, so that new guidelines are needed to attempt to overcome widespread insufficiency, if not frank deficiency.
3. The addition of vitamin D to calcium-rich foods may not only help maintain bone and reduce the risk of osteoporotic factures, but it may also help lower the risks of several cancers and a few other diseases or conditions. Because the recommended intakes for vitamin D of the IOM are now considered to be too low, populations living in northern (or southern) latitudes away from the equator may be at higher risk because of insufficient skin biosynthesis and may be consuming inadequate intakes of this fat-soluble vitamin, even though it is typically fortified in milk and a few other dairy products.
4. Emphasis on the consumption of foods rich in calcium and vitamin D, whether fortified with either nutrient or not, means that animal products must be a major contributor of these nutrients because plant foods typically are low in calcium, except for calcium-fortified soy milk. Strict vegetarians or vegans living at northern or southern latitudes are not likely to be able to synthesize enough vitamin D in their skin for more than six months each year throughout their lives.
5. Concern about excessive calcium intake from all sources, foods and supplements, remains when the UL of intake is set at 2400 mg per day because some individuals are close to or exceeding the UL in the United States. Calcium overload has potentially adverse effects on several organs because of the likelihood soft tissue calcifications.
6. For colonic cell health, adequate calcium and vitamin D intakes may need to be assured along with adequate folate for helping maintain the vitamin D hormone in colonic mucosal cells.

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Section XIII

Meat and Meat Products

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

57

Thermal Processing

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57.1 Introduction 218
57.2 Consideration for Thermal Process Calculations 218
57.2.1 Food Heterogeneity 218
57.2.2 pH 218
57.2.3 Water Activity (a_w) 219
57.2.4 Redox Potential (Eh) 219
57.2.5 Thermal Conductivity 219
57.2.6 Antimicrobial Agents 220
57.3 Enzyme Inactivation and Nutrient Destruction 220
57.4 Heat Transfer Mechanisms 220
57.4.1 Conduction 221
57.4.2 Convection 221
57.4.3 Radiation 222
57.5 Heat and Mass Transfer 222
57.6 Time–Temperature Calculations 223
57.6.1 D-value 223
57.6.2 z-value 224
57.6.3 F-value 224
57.7 Thermal Processing in the Food Industry 225
57.7.1 Scalding 225
57.7.2 Cooking 225
57.7.3 Pasteurization 226
57.7.4 Sterilization 227
57.7.4.1 Canning 227
57.7.4.2 Food Alterations During Canning 229
57.7.4.3 Aseptic Processing 229
References 230

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

57.1 INTRODUCTION

Thermal processing has been applied to foods as a traditional method to achieve several objectives, such as sanitation, preservation, palatability, and improved digestibility. From the sanitation point of view, heat processing is still the cheapest and most efficient method to ensure pathogen-free foods. Preservation is achieved by the destruction of spoilage microorganisms and enzyme inactivation that otherwise lead to food deterioration. Depending on the expected shelf life, a given treatment severity will be applied. Therefore, heat treatment varies from cooking, a relatively mild treatment, to commercial sterilization, a drastic process ensuring that practically all microorganisms are destroyed. Under certain circumstances, toxins produced by some microorganisms are also destroyed. Palatability, considered as consumers' acceptance, is also improved in cases such as meat and fish cooking, vegetable steaming, or bread baking. However, it is important to note that while heat processing improves food chemical and physical characteristics, it frequently, has a deteriorating effect.

57.2 CONSIDERATION FOR THERMAL PROCESS CALCULATIONS

Thermal process calculations derive from basic information: thermal resistance of a given microorganism, where destruction is taken as a basis for calculation, the temperature history of the food, the way it has been handled, and the expected shelf life under given storage conditions. However, other parameters must be considered such as physical and chemical properties of the food; temperature gradient between the food and the heating medium; heat transfer coefficients of food and container; and physical changes in the food during the process (gelling, viscosity reduction or increase, emulsion stability, etc.).

57.2.1 Food Heterogeneity

Microbiologically, to obtain a safe product is to reduce microbial-initiated reactions. To reach this objective, two important considerations are: minimize initial microbial populations and potential microbial colonization.

Physical structure determines the rate and extension of microbial colonization and, therefore, the required severity of any thermal process. Although microorganisms frequently cause spoilage in the food surface, migration to inner parts of the food depends on the food structure. Therefore, knowledge of food microbial ecology is necessary to design an efficient process. Food heterogeneity promotes diversity of habitats, and in consequence, of microbial populations. Macroscopic gradients are common in foods; for instance, lactic acid concentration in Emmenthal cheese (60 μmol/g in the surface and 0–10 μmol/g at the center), is related to the presence of propionibacteria in the center but is absent in the surface, whereas oxygen and pH gradients in Brie cheese determine bacterial population type: the center is anaerobic and highly acidic, and the surface is alkaline and aerobic (Board and others 1992). In tomatoes or tomato paste, if infected by *Alternaria*, *Rhizoctonia*, or *Fusarium*, surface pH is close to 8, but drops to 4.5–4.6 in the inner parts, where healthy tissue is located (Gould 1992).

57.2.2 pH

Foods can be classified, according to their pH, into highly acidic (pH $<$ 3.7), acidic (pH 3.7–4.5) and moderate acidic or neutral (pH $>$ 4.5). pH 4.5 is the grow limit

for *Clostridium botulinum*, a strict anaerobe widely distributed in nature and able to colonize almost any canned food. In low acidic foods, *C. botulinum* can grow, and produce toxins and thermo resistant spores, but cannot grow nor produce toxins in highly acidic foods; destruction of this microorganism is often the calculation basis of thermal processes of foods. Therefore, moderate acidic foods require more severe treatments due to possible pathogen presence (Peck 1997). Thermal process calculations at this pH are also based on inactivation of a facultative anaerobe, *Bacillus stearothermophilus*, its spores are 20 times more resistant than *C. botulinum* spores. Germination of *B. stearothermophilus* spores produce acid flavor due to acid and gas production, its optimum grow temperature is between 49 and 55°C, but cannot survive below 38°C.

A process calculation in acid foods is based on destruction of facultative anaerobes such as *Bacillus coagulans* (*B. thermoacidurans*), *B. macerans*, and *B. polymyxa*. Several thermoresistant spores can survive thermal processing, but if low pH is also a barrier, they do not represent a sanitation problem. Spore forming bacteria do not grow at $pH < 3.7$ (International Commission on Microbiological Specifications for Foods 1998); thermal processing of highly acidic foods is based mainly in yeast and fungi destruction.

57.2.3 Water Activity (a_w)

The food system becomes more or less susceptible to deterioration, dependent on two important factors:

- Amount of available water it contains.
- Amount of water necessary for microbial metabolism and chemical or enzymatic reactions.

Bacteria in general need more than $a_w = 0.90$ to grow; yeast can grow at $a_w > 0.88$ and fungi at $a_w > 0.75$. However, certain bacteria such as the pathogen *Staphylococcus aureus* can grow at $a_w = 0.86$ (Masana and Rodríguez 2006). *C. botulinum* grows at water activity as low as 0.95. Dry products do not need thermal processing, although yeast and fungi tolerate environments with low water activity (International Commission on Microbiological Specifications for Foods 1980).

57.2.4 Redox Potential (Eh)

Redox potential (Eh) is the oxidation–reduction balance, resulting from the concentration of chemical compounds promoting oxidation or reduction in a system. It is directly related to the type of microorganisms present. Strict aerobes, such as *Pseudomonas* can only grow at positive/oxidized Eh (+500 to +100 mv), whereas facultative such as *S. aureus* can grow in a wider range (+30 to −200 mv), and strict anaerobes at negative-reduced Eh, since they do not produce catalase or peroxidase necessary to eliminate preoxidized toxic substances. Chemicals such as proteins and S-containing amino acids are also efficient in promoting a reducing environment (Masana and Rodríguez 2006).

57.2.5 Thermal Conductivity

Heat penetration in foods depends on factors such as chemical composition, physical structure and physicochemical characteristics. Thermal conductivity (Cp), the thermal

flow rate within the product, largely depends on food composition. Carbohydrates, fats, and protein protect microorganisms against heating due to the low heat transfer coefficients of these chemical compounds. Meats, for instance, have very low coefficients (1.89 kJ/h m°K) (Stiebing 1992); if compared to stainless steel coefficient (59.47 kJ/h m°K) (Green and Maloney 1997). It is also important to note that food structure plays an important role in thermal conductivity. If a thermal flow is applied perpendicular to the meat fibers, Cp = 1.72 kJ/h m°K with 78% relative humidity at 0°C. If a thermal flow is applied parallel to the meat fibers, Cp = 1.76 kJ/h m°K at the same relative humidity and temperature conditions (Pérez and Calvelo 1984).

57.2.6 Antimicrobial Agents

Addition of antimicrobial agents is another method to increases sanitation and shelf life in several foods (Leistner 2000). Added benzoates or sorbates as well as chemicals naturally present in the food such as organic acids, essential oils, medium length chain fatty acid, alkaloids, and fitoalexins acts as antimicrobials (Deng and others 1999). Gram-positive bacteria, micrococcus, and *Bacillus* sp. are sensitive to egg conalbumin. Essential oils of cinnamon, garlic, onion, rosemary, and oregano inhibit various yeast species as well a mycelia of several fungi (Draughon 2004).

57.3 ENZYME INACTIVATION AND NUTRIENT DESTRUCTION

Another heat processing objective, in addition to producing foods free from pathogens and spoilage microorganisms, is enzyme inactivation since many spoilage processes are basically of enzymatic nature. Enzyme inactivation depends upon mechanisms similar to those affecting microbial inactivation rates; in fact, microbial destruction is based on inactivation of, at least, one enzyme involved in metabolic pathways (Ashie 2003). However, some isoenzymes are heat resistant and can cause deleterious changes in certain foods; for instance, peroxidases can promote off-odors in peas (Whitaker and others 2003). When thermal processes are calculated for enzyme inhibition, inactivation of the most heat stable enzyme is taken as the calculation basis. At low temperatures, destruction of heat resistant spores is higher than spore inactivation; the opposite is true at high temperatures (Brown 1982). As a result, the food can undergo spoilage due to enzymes that are still active after spore destruction; therefore, the process must be calculated on the basis of enzyme inactivation, not spore destruction. In general, enzyme inactivation and spore destruction take place at the same time: between 130 and 145°C (Dziezak 1991).

57.4 HEAT TRANSFER MECHANISMS

Inactivation of either pathogen or spoilage-causing microorganisms is calculated by heat penetration. Vegetative cells are destroyed at temperature slightly higher than optimum growth temperatures, whereas spores can survive at higher temperatures (Zamudio 2006). As heat application involves the destruction of at least one microbial enzyme,

vegetative cells and spores are inhibited according to a first order reaction rate equation (Baranyi and Roberts 1995):

$$-\frac{\mathrm{dc}}{\mathrm{dt}} = \mathrm{kc}$$

That is, cell concentration (dc) decreases (hence, negative) with time (dt) in a direct proportion of viable cell concentration. However, this is a logarithmic reduction; it decreases in log cycles (for instance, 10^3 to 10^2) as time linearly increases. Using this equation it is possible to compare death rate of several given microbial populations due to the fact that all microorganisms follow the same logarithmic destruction rate (Han 1975).

Thermal processing is basically an operation where heat flows from a hot body (the heating medium) to a cold body (the food). A dynamic process, it is a mechanism where heat flows in proportion to the causing force, and inverse to flow resistance (Karlekar and Desmond 1985). From the thermodynamic point of view, thermal processes are lead by at least one of the following heat transfer mechanisms: conduction, convection, and radiation.

57.4.1 Conduction

Heat is transmitted within a body due to vibration of adjacent molecules following the Fourier law:

$$q = k(A\Delta T/L)$$

where:

A = area
ΔT = temperature difference
L = material thickness
k = material thermal conductivity.

This mechanism occurs is solids, such as pieces of canned food, or material gelling in the can (luncheon meats, desserts, baby foods, etc.) (Mittal and Blaisdel 1984).

57.4.2 Convection

This mechanism takes place in fluid foods due to movement of different parts of the stream having different densities: changes in density occur during heating or cooling. This mechanism follows the Newton law:

$$q = hA\Delta T$$

where:

A = area
ΔT = temperature difference
h = depends on flow properties surface type and flow velocity of the heating medium.

The coefficient, h, varies widely, for instance: gases (natural convection): $h = 2.5-25$ kcal/h m$^{2\circ}$K; water (forced convection): $h = 500 - 5000$ kcal/h m$^{2\circ}$K; condensing steam: $h = 5000 - 15{,}000$ kcal/h m$^{2\circ}$K (Green and Maloney 1997).

Heat transfer during canning of foods such as soup, sauces and brines occurs by this mechanisms; the heat flow direction is from the heating medium (hot water or steam) through a barrier (the can) to a cold fluid within the can (the food) (Welti-Chanes and others 2003). Heat diffusion is faster if an external force is applied, such as can rotation, decreasing temperature difference to a minimum (Valiente 1997).

57.4.3 Radiation

Heat is transmitted by electromagnetic waves from a hot body and absorbed by a cold body. Infrared waves ($\lambda = 0.8 - 400$ μm) are used as a heating medium as they are easily absorbed and transformed into heat (Hanson 1990). This mechanism is seldom used in food processing, but it is commonly used in food preparation just before consumption at home and in hotels, restaurants, and so on.

57.5 HEAT AND MASS TRANSFER

As foods are complex systems, more than the above described mechanism frequently takes place. In addition, simultaneous heat and mass transfer often happens. This occurs in processes such as meat smoking or oven cooking. In meat products such as smoked sausages, moisture permeable casing acts as a barrier between the heating medium and the meat batter; heat is transferred from the hot air in the smokehouse together with chemicals in the smoke to the casing surface; from there, smoke components diffuse though the casing and into the meat batter (Müller 1990). Since the flow rate depends on the temperature difference between the gas environment in the smokehouse and the meat batter, the flow rate increases with the temperature difference, hence increasing the heating rate. Of the three heat transfer mechanisms, conduction and convection are predominant in foods. As conduction occurs by direct contact from particle to particle, this mechanism dominates inside the product from the surface inwards, in a transient state; that is, temperature in any one part of the product will change with time (Valiente 1997). From the heating medium (hot smoke) to the sausage surface, there is a convection mechanism caused by temperature gradients that promote free convection due to density gradients. Heating is more efficient in forced convection systems; this is the reason why industrial smokehouses are fitted with fans. Free convection in air (2.5–25 kcal/h m$^{2\circ}$K), such as oven cooking, if the heat transfer coefficient in the food surface is very small, the limiting factor is convection from the heating medium to the food surface. Conversely, when a very high heat transfer coefficient such as condensing steam (5000–15,000 kcal/h m$^{2\circ}$K) is applied; in this case the limiting factor of conduction rate inside the product (Hanson 1990).

In canning operations, it is assumed that in convection-heated foods all solid pieces immersed in a liquid have the same treatment as the brine. However, this assumption is only valid with solid pieces with high heat transfer coefficients. Since in most cases, heat transfer coefficients are low, process calculation is the same for conduction and convection regimes (Barbosa-Canovas 2003). In cases of a fluid product with severe mixing, such as HTST pasteurization, the same lethality takes place in every point of the food, although conduction cannot be considered.

The extent of heat penetration depends on the dominant mechanism; more intense penetration occurs in low viscosity foods or those with small particles because the main mechanism is by convection. In canned foods penetration can be increased by rotation, generally applied in continuous autoclaves. Very viscous or solid foods are heated by conduction, a slower mechanism occurring in vegetable puree, canned beans, baby foods, or canned sausages (Guerrero Legarreta 2001).

Heat transfer mechanisms may change certain foods during processing. For example, foods containing large amounts of starch or protein that gel within the can, such as dairy desserts, soups, or luncheon meats. During gelling, macromolecules such as proteins or carbohydrates interlink trapping water in the formed network. Due to the high molecular weight of proteins and carbohydrates, they behave as particles in suspension (Li-Chan and others 1985). At initial heating stages, the dominant mechanism is convection caused by density gradient; as particles interlink, heat is transferred by vibration between molecules changing the mechanisms to conduction (Himmelblau 1997). The practical aspect of phase changing is that heating rate also changes. Therefore, in food materials where this gelling is expected to occur heating time should be calculated to avoid overheating.

57.6 TIME–TEMPERATURE CALCULATIONS

Thermal processing calculations are based on the destruction of pathogens, spoilage microorganisms and enzymes. Chemical, physicochemical, and structural food characteristics of the food are taken into consideration, as well as expected shelf life, further storage conditions, packaging material, and quality deterioration. However, the main calculation basis is a given food microorganism. It was already discussed that, in general the strict anaerobe *C. botulinum* is taken as the target microorganism due to its pathogenicity; however, other target microorganisms are *B. stearothermophilus* as the facultative anaerobe. *B. thermoacidurans*, *B. macerans*, and *B. polymyxa* destruction is considered for acid foods, whereas *Coxiella burnetti* is the target microorganism for milk pasteurization (Guerrero Legarreta 2004).

In all thermal processes, as destruction follows a logarithmic model microbial population decrease is asymptotic to the x-axis. The concept of commercial sterility is then applied. Because commercial sterility must be achieved in every part of the food, process evaluation is based on the point taking the longest time to reach process temperature, that is, the "cold point." In conduction mechanisms, the geometric center is at the same time, the cold point. In convection mechanisms, it is located along a vertical axis, around one-third of the distance from the container's bottom end (Guerrero Legarreta 2001). Calculating thermal processes for a new product must include the cold point localization by placing thermocouples in several parts of the container. Several calculated parameters help to control the process efficiency, these parameters are described below.

57.6.1 D-value

Microbial destruction is logarithmic, 90% of the microorganisms are destroyed in a given time at constant temperature. This interval, different for each microorganism, is called decimal reduction time or D-value, it represents the number of minutes necessary to destroy 90% of a given microbial species at a given temperature. The temperature at

which D-value is calculated is written as $D_{70^\circ C}$; D-values are expressed in minutes. For instance, $D_{110^\circ C}$ for *Clostridum sporogenes* (i.e., to reduce 10^5 to 10^4 cells if heated at 110°C) is 10 min ($D_{110^\circ C} = 10$ min) (International Commission on Microbiological Specifications for Foods 1980).

57.6.2 z-value

z-value is the temperature increase necessary to obtain 1 by 10 reduction in D values. For instance, z-value for *C. botulinum* type A is z = 10°C, and $D_{121^\circ C} = 0.2$ min; this means that the same destruction is achieved at 131°C in 0.02 min and at 111°C in 2 min (International Commission on Microbiological Specifications for foods 1996). z-values increase with microbial heat resistance; for yeast, fungi, and bacterial vegetative cells values range from 1.1 to 5.5°C, and between 5.5 and 14°C for spores (International Commission on Microbiological Specifications for Foods 1980).

57.6.3 F-value

Adding up all destruction effects acting upon a microbial population, gives the F-value of the overall process. F-values allow comparing thermal treatments among different foods. F = 1 means the total lethal effects acting during 1 min at 121.1°C (250°F). Known Z-values are used to compare heating times at temperatures above or below 121.1°C. If Z-value is 10°C, it means that the process must be carried out at 111.1°C for 10 min to achieve the same lethal effect, or at 131.1°C for 0.1 min. The relationship between D and F, taking into consideration the initial and final microbial cell concentrations is:

$$F = D(\log a - \log b)$$

where:

a = initial microbial cell concentration

b = final microbial cell concentration.

It is assumed that a moderate acid food (pH > 4.5), is heated to a temperature that ensures that the food is safe from *C. botulinum* contamination. In this case, spore counts must be reduced from 10^{12} to $1 \times 10^{\circ}$, that is, a 12-log cycle reduction, or 12D. In other words, a contamination of one *C. botulinum* spores per gram of food, or $1/10^{12}$. Population of *C. botulinum* type A and B are the calculation basis for D-values at 121.1°C and 0.21 min. Under the 12D concept, foods should be heat treated at F = 2.5, which is called "botulinum heating," to ensure that *C. botulinum* is practically absent (Mathlouthi 1986).

As F-value is the effect of every part of the process, a simple but precise method to calculate the lethal effect during the heating and cooling operations is to record with thermocouples the temperature in the "cold point" of a food or food container, and to calculate the corresponding F-value. The addition of all F-values will be the total F-value for the process.

In the food industry, specific F-values used are defined and calculated, according to specific conditions:

F_s: addition of all lethal effects in an infinite number of points in the container. This is a calculated value, obtained as a result of F-values from all parts of food in the container.

F_c: addition of lethal effects (F) in the geometric center of a food container; that is the coldest point of the food container. F_c is lower than its corresponding F_s because

the heating effect in the containers geometrical center is lower that the total of every part of the container.

57.7 THERMAL PROCESSING IN THE FOOD INDUSTRY

Conversely with microorganisms and enzymes, the aim of a given thermal process is minimal nutrient destruction; since the same factors inhibiting microbial populations also affect nutrients. In some cases, such as HTST processing, temperature increase may cause microbial inhibition but not nutrient deterioration (Mathlouthi 1986).

Heat transfer principles are applied to all thermal processes in foods. However, every process type has a specific objective; process severity depends on this objective. There are basically four types of thermal processes in foods, depending on the applied temperature: scalding, cooking, pasteurization, and sterilization.

57.7.1 Scalding

Scalding is generally applied to fruits and vegetables before freezing, drying, or further processing; therefore scalding objectives depend on the following process. If it is applied before freezing or dehydration, the main objective is enzyme inactivation. Non-scalded frozen or dried food material undergoes changes in color, flavor, and nutritional value as a consequence of enzymatic activity. Peroxidase and catalase, widely distributed in plant material, are highly resistant heat resistant. Activity of these enzymes is taken as an indicator of scalding efficiency; if both are inactivated it is assumed that all other enzymes are also inactive (Quirasco and López Murguía 2006). Scalding time to destroy peroxidase and catalase depends on the fruit or vegetable, heating method, and size of the material. For instance, if scalded in boiling water, small beans require 1–1.5 min to inactivate peroxidase and catalase, whereas large beans 3–4 min; broccoli will need 2–3 min, and spinach 1.5 min (Collins and Marangoni 2000). In some cases, enzyme activation is also achieved during scalding. This is the case of pectinmethylstearase in green beans, its activity provides more firmness to the tissue. Fruits are scalded in calcium containing brines, to achieve more firmness by formation of calcium pectinate (Camire 2000).

In addition to enzyme inactivation, scalding has other advantages such as gas removal from the tissue, increase in tissue temperature and material cleaning. If the material is intended for canning, gas removal to reduce oxygen content in the can; provides better vacuum conditions. Most scalding operations are carried out by food contact with hot water or steam for a given time. Scalding can be a batch operation, although generally is continuous; the food on a conveyor is transported through a steam tunnel. Water scalding can also be a batch and a continuous operation; in batch, the food is immersed in hot water (90–100°C) for a given time. In continuous scalding the food is transported in several ways. If conveyor is used, the residence time depends on the velocity of the moving conveyor. The main problem of this scalding method is the need to renew large water volumes to avoid excessive solids in suspension that may cause off-flavors (Watson and Harper 1988).

57.7.2 Cooking

The main objective of cooking is to improve food sensory characteristics and digestibility. The term "cooking" is applied to different processes: oven cooking, grilling, roasting,

frying, boiling, and steam cooking; the way heat is applied depends on cooking type. Dry heat at more than 100°C is applied in oven cooking, grilling and roasting; boiling and steaming is carried out by placing the food in water; frying involves treatments at more than 200°C (Hanson 1990). Whether the process is carried out at high relative humidity, as well as process temperature, several changes can occur. Dry heat is less efficient than humid heat in inactivating vegetative cells or spores (Mathlouathi 1986). Presence of water also modifies the type of reaction taking place among flavor components (Bailey and Eining 1989).

Cooking is an efficient preservation process, if recontamination is prevented. Toxin destruction is also achieved during cooking, such as hemagglutinins in soybeans or enzyme inhibitors in wheat, as they are protein compounds and can be denaturalized during heating (Iwaoha and Brewer 2000). However, loss of nutrients also takes place. For instance, up to 56% folic acid. Thirty-six percent vitamin B6 and 20% vitamin B1 is lost in corn after 8 min (Collins and Marangoni 2000) of cooking.

57.7.3 Pasteurization

Pasteurization and commercial sterilization are basically the same process. They differ in the severity of heat treatment, and therefore the extent of microbial destruction. Whereas pasteurization was originally aimed at destruction of *Mycobacterium tuberculosis*, the bacteria causing tuberculosis, other bacteria having similar D-values, are also destroyed. However, at industrial level, milk pasteurization parameters are based on destruction of *Coxiella burnetti* (20 min at 60°C) a more heat resistant microorganism than *Mycobacterium tuberculosis* (Masana and Rodríguez 2006).

Heat process optimization is fundamental in pasteurization and commercial sterilization, as temperature-dependent processes such as microbial inactivation and enzyme inactivation proceed in a completely different way. In fluid foods such as milk, HTST process result in a maximum nutrient retention; this is because microbial destruction rate is higher than nutrient degradation (Karlekar and Desmond 1985).

Pasteurization kills part, not all, viable cells in foods, therefore it is applied to foods that will receive additional preservation methods to minimize microbial growth. In most cases, the aim of pasteurization is to destroy pathogens, but spoilage microorganisms can survive, making it necessary to apply other, less severe preservation methods such as refrigeration, addition of chemical preservatives (carbohydrates, acids, salts, etc.), packaging, and so on. Several processing conditions can be applied: high temperature short time pasteurization carried out at 71°C for 15 s in fluid milk produced a good quality and sanitary safe product; a low temperature longer process time is also applied (62°C for 30 s) to fluid milk (Ray 1996). Optimization of this process depends on relative microbial destruction rate and quality factors.

Pasteurization of acid foods is based on the destruction of yeast and fungi; in fermented beverages such as wine or beer pasteurization is calculated to destroy native yeasts, preventing fermentation to proceed before a given limit (Zamudio 2006). Since pasteurization is carried out below 100°C it can be applied to solid foods using the same equipment used in scalding. Acid foods or meat products can be easily pasteurized in a water bath; the packaged product is placed in tanks and heated with water; cooling with water is applied at the end of process. In continuous pasteurization packaged products are moved through a heating tunnel by a conveyor (Hanson 1990).

Continuous pasteurization is also carried out using a spray system, especially for beer and bottled fruit juices. In this method, bottled products are transported in a conveyor through heating and cooling zones in a tunnel; these zones are: primary preheating, secondary preheating, pasteurization, precooling, and final cooling. Tunnels are designed on the basis of temperature gradients, controlled by mixing water and vapor, water used in primary preheating is also further used in precooling; finally, cooling is carried our by water spraying or immersion (Mathlouthi 1986). Pasteurization using steam is applied in metal containers, never in glass; when glass bottles are used care must be taken to prevent thermal shock; this can be achieved by keeping pasteurization-preheating temperature gradients around 20°C, and pasteurization-cooling around 10°C (Müller 1990).

Pasteurization of nonpacked liquids is carried out in direct or indirect heat exchangers, parallel plates, or pipe and shell types, although parallel plates are more commonly used. In parallel plate heat exchangers the product is transported in one side of a wall and a heating medium (vapor or water) in the other side. It is a common process to pasteurize milk, beer, and fruit juice. The plates are generally dented to promote a turbulent flow, increasing heat transfer coefficients up to 4000–9000 kcal/h $m^{2\circ}$K in nonviscous fluid such as milk (Valiente 1997). Energy saving is achieved by using the hot pasteurized fluid as preheating medium for the incoming fluid food.

57.7.4 Sterilization

A sterile product is defined as that in which no microorganism is present. Temperatures above those for microbial growth give result in the death of vegetative cells, but spores can survive. Therefore, sterilization processes are calculated based on spore survival. However, "sterilization" is not an accurate term for food heat treatment, since the sterility criteria cannot be applied in a food product, that is, pathogens are destroyed but nonpathogens may be present but inactive; storage conditions do not allow them to grow. In this situation, foods are "commercially sterile," "microbiologically inactive," or "partially sterile" (Guerrero Legarreta 2006). Commercially sterile foods are merchandised in hermetic containers to prevent recontamination; therefore strict aerobes vegetative cells cannot grow.

However, strict aerobes spores are less resistant to heat than spores of facultative or anaerobes. In foods such as canned cured meat, if oxygen is not completely evacuated and a mild heat treatment is applied together with other preservation methods (curing, refrigeration), aerobes such as *Bacillus subtilis* or *Bacillus mycoides* can be present and promote spoilage (Masana and Rodríguez 2006).

There are two methods of commercial sterilization: heating the food after placing it in a container, or heating and cooling the food then aseptically packaging and sealing. The first method is a conventional canning operation, developed by Nicholas Appert in the 18th century; the second method is known as aseptic packaging. Both processes have the same basic principles.

57.7.4.1 Canning. The objective of canning is to destroy certain microbial populations (vegetative cells and spores) and/or enzymes promoting spoilage or harmful to human health. Problems solved by canning, from a sanitation point of view, are prevention of vegetative cells, and spores, ability to grow and produce toxins, and to eliminate or inhibit microbial development.

The canning process basically consists of four operations: food preparation (cleaning, selection, size reduction, scalding, etc.), can filling, air exhaustion from the can, sealing and thermal process (heating and cooling).

The can is filled with a food at relatively low temperature and placed in a retort, air is then exhausted and the retort filled with condensing steam; latent vaporization heat is transferred through the can into the food; under these circumstances, heat transfer at the surface is very high, therefore the only resistance to heat flow is in the product itself (Watson and Harper 1988).

In addition to preventing quality deterioration by food reaction with oxygen, air exhaustion from the headspace also improves heat penetration. When large food pieces are canned, exhaustion during filling and sealing is generally enough to remove the air (Foottit and Lewis 1999). Conversely, pastes tend to incorporate gas during every filling step; it is not carried out under vacuum. In many cases, cooking is carried out during the sterilization phase of canning; therefore, if small gas bubbles are trapped, adequate sterilization may not be reached (Lan and others 1995). Air exhaustion from the headspace also reduces risks of excessive internal pressure increase during heating, resulting in can blowing or deformation. Risks of anaerobe growth is also reduced (Institute of Food Technologists 2000).

Exhaustion is carried out by heating or by steam injection. Heating (75–95°C) induces steam to replace air in the headspace; cans are immediately closed and sealed. Once cooled down, vacuum is promoted by vapor condensation. However, if the headspace is too large there is incomplete vacuum formation. Alternatively, cans are transported in a conveyor where they are heated at 85–95°C, 90% or more of the air is removed from the headspace, depending on the residence time and temperature of the exhaustion tunnel (Mathlouthi 1986).

Thermal processing during canning operations has two cycles: heating and cooling. Time–temperature relationships are calculated according to microbial destruction and enzyme inactivation criteria, as described before. In general, temperatures applied are 60–90°C for fungi destruction; 60–75°C for enzyme inactivation; 115–121°C for bacterial spore destruction (Foottit and Lewis 1999).

Heat penetration depends on a solid–liquid relationship, and of food distribution within the can. In solid foods in brine, such as canned sausages placed along the vertical axis, a convection–conduction mechanism is established; solid material loosely packed is heated faster than a tightly packed material (Guerrero Legarreta 2001). In general, 30% of the can volume should be a fluid (brine or syrup) in order to get good heat transfer. The can is filled with the liquid after placing the solid food; pastes are almost always handed with dispensers, in this case care must be taken to avoid gas bubbles. For heat transferee calculations, a headspace of approximately 0.5% of the can volume must be considered. In addition, efficiency of air exhaustion from the can depends of the headspace volume (Welti-Chanes and others 2003).

Heat treatment is applied in two main equipment types: autoclave or still retort, used in small plants handling all types of containers (metal, glass, flexible pouches) or large operations handling metal or glass containers; and continuous autoclaves. In a still retort, the cans or glass containers are placed inside, the retort is closed and air is exhausted by steam injection (Manev 1983). Temperature is controlled along the processing time, in order to achieve a given heat transfer rate to the containers.

Cooling is carried out by closing the steam injection and feeding in cold water. Pressure difference is controlled to avoid deformation or lid blowing in large format cans. In

continuous retorts, processing rate is higher, and costs are reduced. In addition, as cans are rotated, heat transfer rate is increased, mainly when a convection mechanisms is dominant. In this operation type, cans are conveyed through a pressure seal to retort parts where processing temperature is applied (Guerrero Legarreta 2001).

57.7.4.2 Food Alterations During Canning. Although canning is a very efficient operation, and foods subjected to this process have considerable shelf life extension, some alterations can occur if processing is not carefully controlled. In delays of more than 20 min between filling and sealing, or before processing, microbial growth can take place; the subsequent heat process can inactivate the microorganisms, but gas or other metabolites may be present in the can (Masana and Rodríguez 2006). Microbial alteration can be also be caused by insufficient heat treatment, as a consequence gas production by surviving microorganism metabolism blows or deforms the can, or the food becomes sour. These alterations are indicative of contamination by sporulated bacteria (Zamudio 2006). In this case, time–temperature process conditions must be checked, as well as food initial microbial load and sanitation of water, equipment, handling, and so on. If similar alterations occur in the same place of the retort, it could be due to operation or equipment failure, such as pipes and valves causing insufficient heating or air in the retort (Hanson 1990). However, it can be also due to inadequate can distribution in the retort, probably packed too tightly, causing heating deficiency as steam or water does not reach every part of the can surface (Thumel 1995). Insufficient or slow cooling may promote thermophile growth. If cans are stored in large blocks, cooling is too slow; therefore, cans must be stored in small well ventilated blocks, particularly in tropical areas when relative humidity and temperature are high (Guerrero Legarreta 2006). Microbial contamination also occurs through seals, if cooling water is not of adequate sanitary quality. Recontamination by cocci, sporulated and nonsporulated bacilli after heat treatment (Brown 1982) is one of the main problems, causing can blowing, meaning sealing failure (Manev 1983).

Although can blowing is generally due to microbial contamination, it can be also due to chemical reactions of the food components to packaging materials producing hydrogen or sulfur compounds. The reaction also promotes bluish black spots, drastically altering flavor and odor. One other very common reaction in canned fruits and vegetables, and dairy products is nonenzymatic browning (Mathlouthi 1986).

Finally, physical alterations in cans occur by equipment mishandling such as fast pressure increase, not enough vacuum or excessive filling. Cans, mainly large formats are distorted and seals can break. Blowing of the bottom end is due to insufficient exhaustion. If cans are transported to high altitudes, blowing is likely to occur; every 300 m increase in altitude, 2.5 cm vacuum is lost. Conversely, excessive exhaustion can cause can collapse (Hanson 1990). Excessive filling causes deformation during heating due to food expansion, mainly in foods that undergo cooking in the can such as luncheon meats, or foods containing several ingredients as meat, beans, corn, rice, and so on that notably increase in size during cooking (Watson and Harper 1988).

57.7.4.3 Aseptic Processing. Food sterilization before packaging involves the same principles as pasteurization; differences are in process severity and applied temperatures. Due to the fact that very high temperature is applied in aseptic commercial processing (132–175°C); it can be considered as a HTST condition process (Welti-Chanes and others 2003). Heat exchangers include rough surface, parallel plates, pipe and shell, and

direct steam injection types (Valiente 1997). Other systems have been developed for fluid containing small particles in suspension; however operation efficiency is considerably reduced when larger particles, such as peas in brine, are processed, mainly due to pumping. Direct seam injection has been used for milk sterilization in a process known as uperization; it is efficient only if further refrigeration is applied (Thumel 1995).

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58

Ham

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58.1 Introduction 234
58.2 Traditional Processing of Dry-Cured Ham 234
58.3 Dry-Cured Ham: Modern Processing Technology 234
58.3.1 Reception 235
58.3.2 Presalting 236
58.3.3 Salting 236
58.3.4 Postsalting 237
58.3.5 Smoking 237
58.3.6 Ripening–Drying 237
58.3.7 Extended Ripening 237
58.3.8 Final Product 237
58.4 Physical and (Bio)Chemical Changes During the Processing of Dry-Cured Ham 238
58.5 Types of Dry-Cured Hams 240
58.6 Accelerated Processing 241
58.7 Cooked Ham: Processing Technology 241
58.7.1 Reception 241
58.7.2 Brine Injection 242
58.7.3 Massaging and/or Tumbling 243
58.7.4 Cooking 243
58.7.5 Cooling 244
58.7.6 Final Product 244
58.8 Physical and (Bio)Chemical Changes During the Processing of Cooked Ham 244
58.8.1 Color 244
58.8.2 Texture 245
58.8.3 Biochemical Changes 245
References 246

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58.1 INTRODUCTION

There are two important types of hams which mainly depend on the different processing technology. Dry-cured ham involves the use of a dry cure that is rubbed on the external surface followed by a mild drying. On the other hand, wet-cured ham or cooked ham involves the use of brine that is either injected or soaked followed by the application of thermal treatment. The production of both types of hams are described in this chapter.

Dry-cured ham was originally produced for preservation purposes and as a food reservoir for times of scarcity. The expanded use of refrigeration during the last century reduced this need but increased the need for better consumer acceptance. So, the processing technology was slightly modified in order to obtain better sensory acceptance even though it remained largely empirical, following the experience transmitted by manufacturers from generation to generation (Toldrá and others 1997). Scientific knowledge on chemical and biochemical mechanisms related with flavor and texture development in curing processes experienced a rapid increase during the last decades of the 20th century and prompted some technological evolution. In-depth knowledge on numerous biochemical reactions, mainly affecting proteins and lipids, were thus available. These reactions take place in postmortem meat and are especially significant during the dry-curing process, mainly along the ripening period and have a decisive contribution to the development of an adequate texture and a characteristic flavor (Parolari 1996; Toldrá 1998; Toldrá and Flores 1998). The adequate control of these reactions is essential for quality standardization (Toldrá 2004a; Toldrá and others 2004). Today, there are different technologies depending on the raw materials and the processing conditions. The processing technologies for the production of both types, dry-cured ham and cooked ham, are discussed in this chapter.

58.2 TRADITIONAL PROCESSING OF DRY-CURED HAM

The traditional processing of hams was more usual in old times but today is limited in production and restricted to rural areas located near the mountains where cool and dry winds are available most of the year. Basically, it consists in rearing pigs at home for about 1 year. These pigs are slaughtered by the end of November to coincide with the beginning of the coldest months. Hams are then salted by rubbing salt on their surface and left for a few weeks. Once the salt is diffused through the whole ham, they are hung in big rooms on the top of the house for the ripening and drying processes which coincide with the spring and summer. More or less aeration is achieved by manual opening of windows. The decision is based on subjective evaluation of the hams status. Total processing time takes near 1 year, the hams being ready for the consumption by early autumn.

58.3 DRY-CURED HAM: MODERN PROCESSING TECHNOLOGY

The processing of dry-cured hams is apparently simple but requires a minimum length for the muscle enzymes to develop the characteristic flavor. Main stages are the reception of hams, salting, postsalting, ripening, and drying as shown in Figure 58.1.

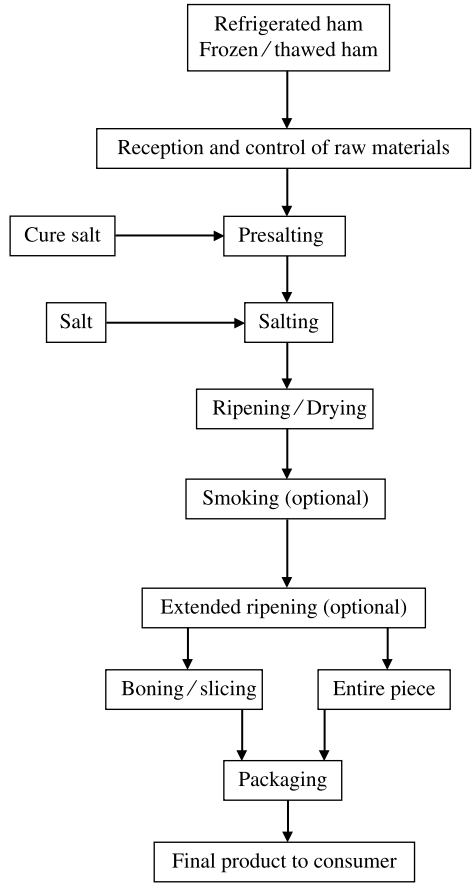

Figure 58.1 *Process flow diagram for the processing of dry-cured hams.*

58.3.1 Reception

This step is crucial because all the process depends on the quality of raw hams. It is important to discriminate DFD (dark, firm, and dry) hams because its high pH can prompt the development of undesirable microorganisms. DFD hams must be used for other types of products. On the other hand, PSE (pale, soft, and exudative) hams create some difficulties to processors because of an excessive rapid water loss due to their lower water-binding capacity (Arnau and others 1995) and an unpleasant salty taste due to an excess of salt intake. Selection by weight facilitates the control of time during the salting stage as will be described later. The control of fat is important for correct flavor development. Fat composition in fatty acids depends on the feed given to pigs (Toldrá 2005; Jiménez-Colmenero and others 2006), and the crossbreed used (Armero and others 2002). The detection of any undesirable oxidation and/or development of rancid off-flavors is essential at this stage. Easy controls for unsaturation and freshness consist in the measurement of the iodine index and the acid index, respectively (Toldrá 2002).

In general, hams from heavy pigs give better quality (reddish color, intramuscular fat, marbling, etc.) than industrial standard pigs. Main reasons are related with chemical and biochemical characteristics of respective muscles (i.e., different enzyme profile) (Toldrá and others 1996).

Frozen hams are allowed to thaw till they reach an internal temperature about 2–4°C. The skin is partially removed, leaving an area where salt will penetrate and water will evaporate. Hams are registered on its surface to facilitate traceability and subjected to pressing rollers for bleeding. Just before salting, part of the skin is removed in order to allow salt penetration and water evaporation. The size of this area depends on the different types of ham.

58.3.2 Presalting

Refrigerated hams or frozen/thawed hams are stored for 1–2 days at 2–4°C to reach a uniform temperature. Then, hams are bled to extract any remaining blood inside and rubbed on the external surface with the curing salt (a mixture of sodium chloride and potassium nitrate) to get a final nitrate concentration of 150 mg/kg inside the ham. In some cases, the curing salt may be directly applied in the salting stage (i.e., for French and country-style hams). Nitrate, that is added as a protective agent against botulism, is slowly reduced to nitrite by the action of nitrate reductase (Cassens 1995), a bacterial enzyme present in the natural flora (i.e., Micrococcaceae) of ham. The European Union allows a maximum amount of 150 ppm potassium nitrate or 300 ppm for the combination of potassium nitrate + sodium nitrite, while the United States allows 156 ppm sodium nitrite ($\frac{1}{4}$ ounce per 100 pounds of meat).

58.3.3 Salting

This stage corresponds to the introduction of salt into the ham. There are several important roles for salt like reduction of a_w and inhibition of the growth of spoilage microorganisms, imparting a characteristic salty taste and increase of the solubility of myofibrillar proteins. Salt is added to the external surface of the hams in an exact proportion, hand-rubbed and left for penetration into the ham. For instance, 20–30 g medium-grain salt per kg on the lean surface and 10–20 g of wet salt per kg on the skin during 14–20 days for Parma hams (Parolari 1996). In other cases, like in Spain, hams are surrounded by dry salt (rough sea salt or refined mineral salt) and time of salting is strictly controlled to 1.1 day per kg

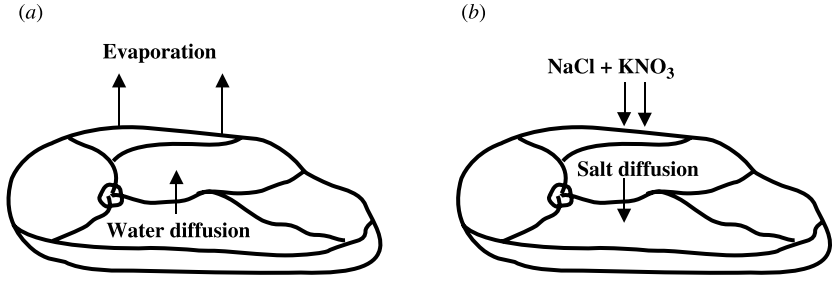

Figure 58.2 *Scheme showing the uptake of salt and nitrate during the salting and then its diffusion during the postsalting stage.*

(Toldrá 2002). Weight losses are about 3–4% and the excess of salt is removed by rinsing and brushing at the end of this stage.

58.3.4 Postsalting

Salt that has penetrated into the ham slowly diffuses to the inner area (see Fig. 58.2*a*). This takes 40–60 days, depending on the size of the ham, pH, amount of intramuscular fat, and temperature. Weight losses may reach around 4–6%.

58.3.5 Smoking

The use of smoke is used in short processes like American country-style or German Westphalia ham. Smoking is more typical in areas where drying was originally more difficult. Consumers are used to the smoked flavor. Furthermore, smoke compounds constitute a means of preservation due to its bactericidal effects (Ellis 2001).

58.3.6 Ripening–Drying

This stage is very important in order to reach the final desired quality. There are many combinations of air speed, temperature, and relative humidity which are typical for each type of ham. This is achieved through the use of computer-controlled drying chambers. Relative humidity must be carefully controlled to avoid excessive evaporation of eater on the surface of the ham (see Fig. 58.2*b*). Ripening and drying are very specific for each type of ham. One of the targets consists in achieving a final weight loss around 32–36% in relation to the initial weight. Then, the length of the drying will depend on when this goal is reached. Usually, at least 6–9 months are necessary. High quality hams are further ripened to allow for more intense enzyme action and flavor development. These hams are covered with a layer of lard to prevent further dehydration. The quality of hams (texture, appearance, color, and flavor) is monitored and evaluated by experts. A sniffing test, consisting in the insertion of a small probe till the bones junction and rapid sniffing for any off-flavor, allows the detection of any spoilage inside the ham (Parolari 1996).

58.3.7 Extended Ripening

Hams of excellent quality, like Iberian hams, may experience further ripening cellars at mild temperatures for long periods of time up to 24–30 months. These hams exhibit exquisite and intense flavors generated through further chemical and enzymatic reactions. Sometimes, the proteolysis is so intense that produces large amounts of free amino acids. Tyrosine, that is quite insoluble, produces small crystals visible as white spots on the cut surface (see Fig. 58.3).

58.3.8 Final Product

The commercial distribution of hams may be as an entire piece, as a boned piece or as slices. Boned hams can also be sliced by retailers or directly by consumers at home. A cross section of a ham where intense marbling can be appreciated is shown in Figure 58.3. Sliced ham may be vacuum-packaged or kept under controlled atmosphere

Figure 58.3 *Section of a typical dry-cured ham. Some small white crystal spots of tyrosine, proving intense proteolysis, can be observed.*

(Toldrá and others 2004). The typical consumption of dry-cured ham is as an entire piece (see Fig. 58.4).

58.4 PHYSICAL AND (BIO)CHEMICAL CHANGES DURING THE PROCESSING OF DRY-CURED HAM

There are two important diffusion phenomena in dry-cured ham affecting water and salt. Water must diffuse through the piece of ham towards the external surface. Then, water is

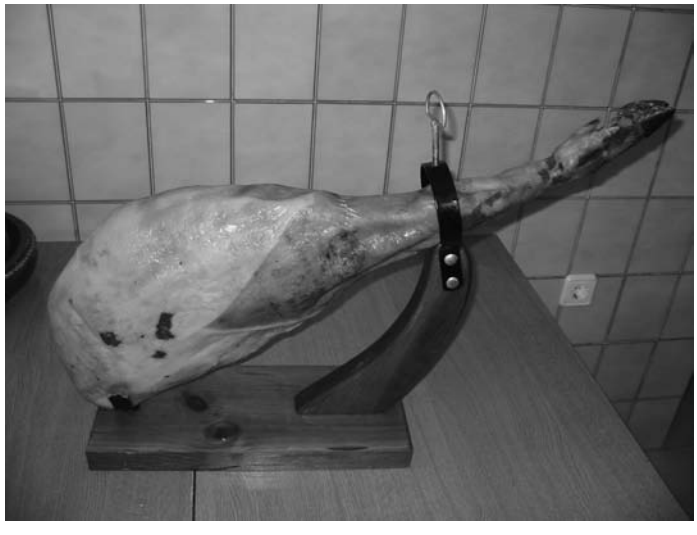

Figure 58.4 *A typical ham ready for slicing.*

evaporated to the environment depending on the relative humidity existing in the drying chamber (see Fig. 58.2*a*). The equilibrium is difficult to reach but is extremely important to get an optimal quality. A slow diffusion would accumulate water into the ham and would give poor weight loss and soft texture. However, an excess of evaporation results in excessively dried hams and tough textures (Toldrá 2005).

Diffusion of salt is a very slow process that depends on many variables like the size of the ham, its temperature, pH, water content, and intramuscular fat. Once salt penetrates into the ham, there is an equalization through the full piece that may take up to 3–4 months (see Fig. 58.2*b*).

The pH range is quite limited for the full process, starting at pH about 5.6 at the beginning to values near 6.4 for the final product. PSE hams have a pH evolution similar to normal ones even though they take more salt due to their initial lower pH and excessive moisture (Arnau and others 1995). All the enzymes and chemical reactions operate within this narrow range (Toldrá 1998) even though more intense proteolysis has been reported in low-pH hams (Buscailhon and others 1994a).

Most of the biochemical changes in dry-cured ham are a consequence of enzymatic reactions (Toldrá 1992). Proteolysis and lipolysis constitute two of the most important enzymatic phenomena, responsible for the generation of compounds with direct influence on taste and aroma.

Proteolysis consists in the generation of peptides and free amino acids from the progressive enzymatic degradation of major sarcoplasmic and myofibrillar proteins. This chain of reactions constitute the basis for important biochemical changes in the ham (Toldrá 2006a). Main muscle proteases are cathepsins B, D, H, and L; calpains I and II; tripeptidylpeptidases I and II; dipeptidylpeptidases I, II, III, and IV, and alanyl, arginyl, leucyl, and methyl aminopeptidases (Toldrá 2005). The activity of these endogenous muscle enzymes depends on the original crossbreeds (Armero and others 1999a,b; Soriano and others 2005) and the age of the pigs (Toldrá and others 1996; Rosell and Toldrá 1998). These enzymes have shown very good stability in long dry-curing processes (Toldrá and Etherington 1988; Toldrá and others 1993; Toldrá and others 2004). Temperature, time of drying, and ripening determine the action of these enzymes and, thus, the final sensory quality. The amount of salt exerts an important inhibitory effect on cathepsins (Rico and others 1990, 1991; Toldrá and others 1992a,b) and peptidases (Sentandreu and Toldrá 2001). In fact, hams with high cathepsin B activity and low salt content may result in soft hams (Parolari and others 1994). A good number of peptides generated as a consequence of peptidases action (Aristoy and Toldrá 1995). Finally, dry-cured hams are characterized by important amounts of free amino acids which are generated by endogenous muscle aminopeptidases (Toldrá and others 1995). The generation of free amino acids is extremely large in dry-cured ham and is somehow affected by levels of salt (Toldrá and others 2000). For instance, lysine and tyrosine are well correlated to an improvement in the aged taste of Parma ham (Careri and others 1993). The levels of salt also affect salty taste and texture of hams (Andrés and others 2004, 2005).

Lipolysis consists in the enzymatic generation of free fatty acids from the breakdown of triacylglycerols and phospholipids. Main lipolytic enzymes are lysosomal acid lipase and acid phospholipase that are located in muscle and neutral lipase located in adipose tissue (Toldrá 2005) and have also shown good stability through the entire process (Motilva and others 1992; Toldrá and Navarro 2002). The activity of these enzymes also depends on the type of breeding (Armero and others 2002; Cava and others 2004) and age (Toldrá and

others 1996). The generation rate for the release of free fatty acids during the process increases for up to 10 months in the muscle (Motilva and others 2003a; Vestergaard and others 2000) and up to 6 months in adipose tissue (Motilva and others 2003b; Buscailhon and others 1994b).

The generated free fatty acids with one or more double bonds are susceptible to further oxidative reactions that will generate volatile compounds with particular aroma characteristics (Berdagué and others 1991; Buscailhon and others 1993). Muscle oxidative enzymes, like peroxidases and cyclooxygenases, the content in moisture and metallic cations, and other external factors like light and heating, catalyze the generation of free radicals which are very reactive (Coutron-Gambotti and Gandemer 1999). Secondary oxidation products are formed and contribute to flavor (Flores and others 1998). Many volatile compounds with different contribution to ham flavor are generated. For instance, aldehydes and ketones are related to the aroma of French-type hams (Buscailhon and others 1994c) and Spanish hams (Flores and others 1997; Sabio and others 1998) while esters are well correlated with Parma ham aged odor (Careri and others 1993; Bolzoni and others 1996). Alkanes and alkenes as major volatile compounds and branched aldehydes and sulphur compounds have been found in Chinese Jinhua ham (Du and Ahn 2001). Due to the particular generation of volatile compounds according to the type of process, a method based on solid phase extraction of volatile compounds followed by its injection into a gas chromatograph has been recently proposed to characterize aromas (Pérez-Juan and others 2006) or differentiate among types of hams (Luna and others 2006; Pérez-Juan and others 2006). Main sensory characteristics are described in the chapter on sensory quality of meat products.

58.5 TYPES OF DRY-CURED HAMS

There are many varieties of dry-cured hams depending on the raw materials, the region or country of origin, the type of process, the additional smoking, and so on. So, different types of hams are produced throughout Europe. Iberian hams are produced in southwestern areas of Spain through a very long process that may reach up to 2 years or longer. Raw hams are selected from autochthonous Iberian heavy pigs which are bred extensively and fed with acorn and grass, essential for the final characteristic flavor of the product (Toldrá and others 1997; Carrapiso and others 2003; Cava and others 2004). Corsican hams are also produced in Corsica (France) from autochthonous heavy pigs bred in extensive system and fattened with chestnuts. Its processing may take up to 18 months even though the total production is very low. Known hams are produced from certain crossbreeds of white pigs which are slaughtered at about 110 kg live weight or even higher weights like 150 kg for Parma ham. There is a careful selection of the raw materials and processing conditions. Processing time is usually above 12 months. Some of the most well-known are Spanish Serrano, Italian San Danielle, and French Bayonne dry-cured hams. The European Union (EU) gives different labels to protect these hams like Protected Designation of Origin, Protected Geographical Indication, or Traditional Speciality Guaranteed. Hams are then controlled by consortiums (i.e., the Parma Consortium or the Serrano Foundation) that inspect the compliance of all the specific requirements. Hams produced in the Mediterranean area are eaten without further smoking or

cooking (Toldrá 2004b). A picture of an entire piece of ham, ready for consumption, is shown in Figure 58.4.

Traditional hams in northern Europe are usually processed for shorter times and smoked and cooked before consumption. This is the case of the traditional German Westphalian ham, the German Katenschinken (cold smoked ham) and the Finnish "sauna" hams (Puolanne 1982). American hams, known as country-style hams, are also smoked and cooked. Some of the most well-known are produced in Kentucky and Virginia. Hams produced in other areas of the world are also growing significantly in recent years. Good examples are those hams like Ying Hua or Yunnan produced in China (Campbell-Platt 1995; Du and Ahn 2001).

58.6 ACCELERATED PROCESSING

Dry-curing constitutes a long time-requiring process. This is the main reason for the different attemps to accelerate the process. Some strategies are focused on the salting process in order to facilitate the penetration and diffusion of salt into the hams (Marriott and others 1987, 1992). So, some researchers have assayed boning and skinning of hams (Montgomery and others 1976; Kemp and others 1980; Marriott and others 1983), tumbling of hams in rotative drums (Leak and others 1984) or mechanical tenderization through blade penetration prior to dry cure. Other strategies are centered on membrane disruption that can be easily achieved by freezing and thawing of hams (Kemp and others 1982; Motilva and others 1994). More recent attemps have consisted in the substitution of the salting stage by using simultaneous brine salting method while frozen hams are thawed. This process can be further accelerated using simultaneous vacuum impregnation (Barat and others 2006). It gives a substantial reduction in the time needed for thawing and salting without affecting the biochemical reactions taking place during the processing (Flores and others 2006a) nor the sensory quality of the final product (Flores and others 2006b).

58.7 COOKED HAM: PROCESSING TECHNOLOGY

Cooked ham is also known as canned ham. Main stages are the reception of hams, the brine injection, tumbling and massaging, cooking, and cooling as shown in Figure 58.5.

58.7.1 Reception

Refrigerated hams or frozen/thawed hams must be controlled before its processing. In this type of process where hams are cooked but not dried, DFD (dry, firm and dry) hams are not rejected. Its high pH is not a problem because these hams are heat-treated avoiding any risk for microbial growth. Furthermore, the high pH facilitates water retention. As mentioned for dry-cured hams, the fat is very important for flavor development. The composition in fatty acids, that depends on the feed given to pigs (Toldrá 2005; Jiménez-Colmenero and others 2006) and the crossbreed used (Armero and others 2002), will affect the final aroma profile. Any undesirable aroma (i.e., rancidity) or oxidative development must be detected before processing. In most cases, hams are boned to facilitate the brine injection and diffusion.

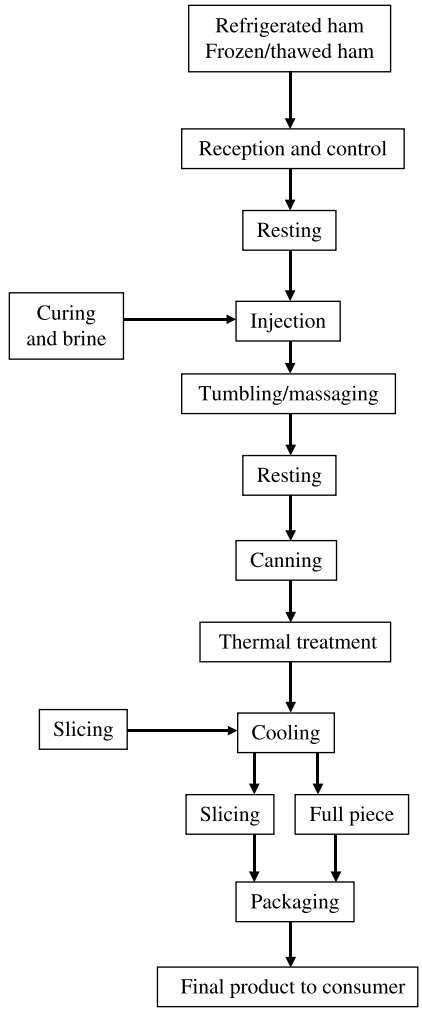

Figure 58.5 *Process flow diagram for the processing of cooked hams.*

58.7.2 Brine Injection

Salt is the main ingredient of brines and its amount varies depending on the type of product. It can be accompanied by sugars like sucrose, dextrose, or corn syrup, to give a pleasant mild taste. It is important that salt has a reduced content of impurities in order to reduce lipid oxidation. Nitrite is added to assure the generation of nitric oxide for preservation and color formation. Sodium ascorbate or sodium erythorbate are added to assure rapid nitrite disappearance and protection against the generation of nitrosamines. Other additives may be added to the brine but this is only used for the production of low quality hams. This is the case of phosphates, polyphosphates, or pyrophosphates that increase the pH of the ham far from the isoelectric point of muscle proteins, which is around 4.5, and thus increase the amount of retained water. Phosphates also increase the ionic strength and contribute to protein solubilization.

Figure 58.6 *Picture of a typical brine injection machine with multineedle.*

The brine is injected into the ham through a multi-needle system (see Fig. 58.6). Pumping speed and volume of injection may be controlled. The injection machines may pump brine into boneless hams or also into bone-in-hams. The brine favors the binding of muscles, the solubilization of protein and gives a better yield and a higher final weight.

After the brine injection it is a current practice to hold the hams for resting during a brief period of time to help the diffusion of salt and additives through the entire piece.

58.7.3 Massaging and/or Tumbling

This stage is also operated under refrigeration conditions. Hams can be massaged for a few hours in mixers under mild agitation to avoid physical disruptions or damages. The paddles of the mixer exert minimal damage under these low speed conditions and preserve the full muscle's appearance. In the case of tumbling, hams are moved in rotary tumblers operating under vacuum to avoid air bubbles and further undesired oxidations. There are many designs for tumblers but, in general, they consist in rotary drums with inner baffles to ensure maximum homogenization of the brine inside the hams. This operation also facilitates improved tenderness and juiciness.

58.7.4 Cooking

Cooking of hams is a delicate stage that requires a rigorous control of time and temperature to achieve the final desired effect and ensure the wholesomeness of the product (Ponce-Alquicira 2005). Heat treatment aims microbial destruction and enzyme inactivation. The heat treatment is calculated to inactivate pathogen and spoilage microorganisms for extended shelf-life of the product with minor effect on sensory characteristics (Guerrero-Legarreta 2001). Hams are canned in special molds that will give the final shape. They can optionally be packaged in special plastic (zero water loss). Cooking can be considered as pasteurization since internal temperature into the ham reaches around 72°C for 30–60 min. Cooking, that can be achieved with either steam or hot

water baths, needs a precise control of temperature. The speed of temperature increase during cooking is important. There are two heat transfer mechanisms: Convection which is the transfer from the heating medium to the ham surface and conduction which is the main heat transfer mechanism from the ham surface to the inner areas (Guerrero-Legarreta 2005). Cooking can be operated in three ways: (1) heating at a fixed temperature but this process may give some problems of excess or lack of heating; (2) heating until reaching a determined temperature inside the ham (usually 68°C), and (3) heating by stages that consist to increase the temperature of the ham through steps, not exceeding 25–30°C each time. This process avoids excessive heating. The cooking process contributes to the final development of sensory characteristics typical of these hams. Several reactions, like enzymatic reactions, oxidations, Maillard reactions, and so on, take place into the hams during this stage (Toldrá 2006b).

58.7.5 Cooling

Cooling is also a delicate stage to ensure the wholesomeness of the hams and complete food safety. Cooling is achieved by inmersion in cold water or with cold water showers. The target is to achieve temperatures of the ham down to 5°C. However, the most hazardous period is the transit between 50° down to 12° where bacteria might grow, especially under slow cooling conditions. Total time to decrease temperature to 5°C should not exceed 10 h. The rate for the cooling process depends on the number of spores forming bacteria.

58.7.6 Final Product

Once hams are cooled, they are taken out of the molds and packaged. At this stage, hams can be given a surface heat treatment to avoid any recontamination before packaging. In some cases (smoked hams), hams can be smoked to acquire a typical color and smoke flavor (Ellis 2001). Cooked hams are usually commercialized in slices ready to be consumed. A wide variety of vacuum packages containing different numbers of slices are typically found in supermarkets. More recently, other types of packages like those using modified atmosphere are available. Hams can also be sold to retailers as a full piece for direct slicing to consumers under request.

58.8 PHYSICAL AND (BIO)CHEMICAL CHANGES DURING THE PROCESSING OF COOKED HAM

58.8.1 Color

The color of ham mainly depends on the concentration of its natural pigment myoglobin. Its content depends on the type of muscle and the age of the animal, being higher in older animals and in muscles with oxidative pattern (Laborde and others 1985; Aristoy and Toldrá 1998).

The typical color of cooked ham changes from red to pink during the heating process, especially at temperatures above 65°C. This is due to the transformation of nitrosylmyoglobin, a compound formed after reaction of nitric oxide with myoglobin, into nitrosylhemocrome which has a typical light pink cured meat color. Upon heating, globin denatures and detaches

itself from the iron atom, and surrounds the heme moiety. This color is also known as cooked cured-meat pigment (Pegg and Shahidi 2000). Some darker surface colors on smoked hams may result from the pyrolytic decomposition of wood.

58.8.2 Texture

The texture of cooked hams depends on several factors, such as the extent of heating (structure breakdown), the moisture content, the extent of proteolysis (degree of myofibrillar protein breakdown), and the content of connective tissue. The content of intramuscular fat also exerts a positive influence on some texture and appearance traits. Starches added to ham help to enhance texture and bind water as well as improve the mouth feel. There are different types of starches and each one will impart different texture and sensory properties to the cooked product. So, starches may be extracted either from cereals (i.e., wheat or corn) or roots and tubers (i.e., potato). Commercial starches are usually modified to pregelatinization to be cold water swellable, cross-linking to impart stability for cooked ham processing, and stabilization to increase the water holding capacity (Martin 2001).

58.8.3 Biochemical Changes

Cooked ham experiences some biochemical changes as a consequence of enzymatic reactions, mainly through proteolysis and lipolysis. Muscle proteases and lipases contribute to the generation of free amino acids and fatty acids with influence on taste and aroma even though these enzymes have a reduced time for action. A comparison of main reactions in cooked ham to those in dry-cured ham is shown in Table 58.1.

The conditions into the hams (high water activity, low salt content) favor the proteolysis but these enzymes get rapidly inactivated during cooking because they are sensitive to temperatures above 50°C where their stability decreases rapidly (Toldrá and others 1992a, b). In any case, there is generation of free amino acids by muscle aminopeptidases that contribute to taste (Flores and others 1998) but the amount of released amino acids depends on the extent of resting before cooking.

TABLE 58.1 Main Groups of (Bio)chemical Reactions Affecting Sensory Properties of Dry-Cured and Cooked Hams.

Groups of Reactions	Dry-Cured Ham	Cooked Ham
Protein degradation	Intense by enzymatic proteolysis	Intense by heat denaturation
Generation of small peptides and free amino acids by proteolysis	Large	Poor
Lipid degradation	Intense by enzymatic lipolysis	Medium by heat damage
Generation of free fatty acids by lipolysis	Large	Poor
Oxidation of free fatty acids	Intense	Medium
Generation of volatile compounds	Intense	Medium
Strecker degradation of amino acids	Intense	Scarce
Maillard reactions	Medium	Intense
Cured color generation	Nitrosomyoglobin	Nitrosohemocrome

Lipolysis is also favored by conditions previous to cooking, especially the pH near neutral conditions. Fatty acids are released during resting and initial cooking of hams (Toldrá 2006b). One or two days of resting, previous to cooking, allows longer enzymatic action and larger amounts of released amino acids and fatty acids that will act as substrates for further chemical reactions (i.e., Strecker reactions) responsible for the generation of volatile compounds. As in the case of proteases, lipases are also inactivated during cooking. The composition in fatty acids is a key aspect in flavor generation. An excess of linoleic acid may impart some off-flavor during cooking. Further chemical reactions (i.e., Maillard reactions) are accelerated during cooking and contribute to the generation of aroma volatile compounds. The extent and characteristics of flavor will depend on the time and intensity of heating.

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59

Sausages

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59.1 Introduction 252
59.2 Classification of Sausages 252
59.3 Types of Sausages 253
59.3.1 Fresh Sausages 253
59.3.2 Raw Fermented Sausages 253
59.3.3 Semidry Sausages 254
59.3.4 Dry Sausages 254
59.3.4 Cooked Sausages 256
59.4 Ingredients and Additives 256
59.4.1 Meat and Fat 256
59.4.2 Salt 256
59.4.3 Nitrite and Nitrate 256
59.4.4 Carbohydrates 257
59.4.5 Spices and Flavorings 257
59.4.6 Starter Cultures 257
59.5 Processing Technology 257
59.5.1 Comminution and Stuffing 258
59.5.2 Emulsification 260
59.5.3 Fermentation 260
59.5.4 Smoking 260
59.5.5 Ripening/Drying 261
59.5.6 Cooking 261
59.5.7 Packaging 261
59.6 Sensory Characteristics 262
References 262

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

59.1 INTRODUCTION

The production and consumption of sausages, in particular those produced through drying, probably originated around the Mediterranean area, as its particular climate allowed a natural drying and ripening process, and these were intense commercial exchanges. On the other hand, the use of smoke was applied in northern and colder areas where the climate did not allow a natural drying.

There are many historical references to pork and its products. Pork was described as an important food source in Sumer, several times, in numerous Sumerian tablets, written in the cuneiform language around the year 2000 B.C. (Kramer 1965). In ancient Egypt, Seth was introduced into the the eye of Horus in the form of a black pig, blinding him (Max Müller 1996). Ancient Romans and Greeks also produced and consumed numerous pig products, coinciding with the great expansion of the pig throughout Europe. Specific details of pig slaughtering and the cooking of its products were described ca. 900 B.C. in the famous Odyssey (Homero 1993). In the first century, Petronio described in his book "El satiricón" the famous banquet of Trimalción, where one of the main dishes consisted of products from wild pig (Petronio 1965). Romans found that salted meats were easy to store and/or transport, assuring a food supply at distances from home. It is not surprising that some expressions like the Spanish "salchichón" or the French "saucisson" were probably derived from the latin word "salsicia" or the Italian "Salami" from the latin word "salumen". On the other hand, the word salami has also been attributed to the city of Salamis in Cyprus (Zeuthen 1995). Recipes of salted meat products, which are still consumed in certain Mediterranean areas, were described by Catón in his "De Re Agricola" (Pineda 1989). Roman coins adorned with the shape of a pig leg are to be found in museums. In the fifth century, the French butchers known as "charcutiers" already prepared different pork derivatives. The Saint Antoine l'Abbaye practiced innovative medicine in the twelfth century using pork meat and lard. Pork meat products were widespread in consumption during the Middle Ages. A good number of pork meat products were described in the famous book "El Quijote" written by Miguel de Cervantes in 1604. As an example, Dulcinea del Toboso was described to have better hands for pork salting. Chinese cooked sausages like lup cheong have been known in China for more than 2500 years.

The modern dry-fermented sausage was apparently invented around 1730 in Italy, being later adopted around 1780 by the German countries after the stay in Italy of a German butcher named Butleb (Leistner 1992). Manufacturing procedures had to be adapted to the climatic conditions of the production area. As an example, the summer sausage was produced in the summer and was heated for safety reasons (Zeuthen 1995). Manufacturing practices were brought to America by settlers and many typical European sausages may still be found in Northern States like Wisconsin.

59.2 CLASSIFICATION OF SAUSAGES

The term "sausage" is used for a great number of meat products, although its meaning may vary depending on the kind of product and country of origin. Traditionally, this term means a product consisting of a mince of ground meat and fat, which is stuffed into a casing. It also involves the use of a curing salt (sodium chloride + nitrate/nitrite) that contributes to its characteristic color and flavor. Thus, it is a broad term applied to many different meat

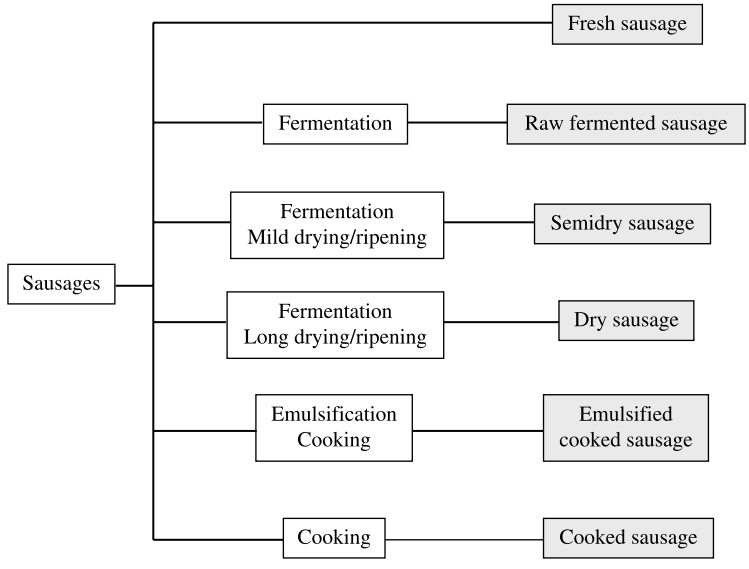

Figure 59.1 *Scheme for classification of sausages.*

products. Basically, sausages can be arranged into several groups based on the main preservation practices of fermentation, drying, or cooking (Flores and Toldrá 1993). A general scheme for this classification is shown in Figure 59.1. Depending on the conditions used for fermentation, the sausages can be low-acid or high-acid (Incze 2004). In the case of drying, there are further classifications depending on the fermentation, time for ripening, and extent of drying. So, taking into account the effect of drying and the remaining moisture content, sausages may be classified as semidry when the weight loss is lower than 20% or dry when the weight loss exceeds 30%. The length of the ripening period depends on the kind of product (size and diameter) as well as the desired final quality. The process can be considered as rapid when it takes less than 7 days, regular when it takes around 3 weeks, or slow when it exceeds 90–120 days (Toldrá 2002).

59.3 TYPES OF SAUSAGES

59.3.1 Fresh Sausages

Fresh sausage may also be known as moist sausage, because its moisture content is high, at between 50 and 60%. These sausages are stored for a few days under refrigeration and are cooked before eating. Fresh sausages can differ in texture, seasoning, type of casings, and meat content (Ockerman and Basu 2004). Some of the most well known are mettwurst and bockwurst, which were originally fresh, but more recently are cooked (see Table 59.1). Others, like country-style sausages, may be smoked.

59.3.2 Raw Fermented Sausages

These sausages are fermented at high temperatures so that lactic acid bacteria may grow and drop the pH towards acid values. Merguez-type sausages are produced in North

TABLE 59.1 Examples of Different Types of Sausages.

Product Type	Moisture	Treatment	Denomination
Fresh sausages	Moist	May be cooked	Mettwurst
Fresh sausages	Moist	May be cooked	Bockwurst
Fresh sausages	Moist	May be smoked	Country-style
Fresh sausages	Moist	No treatment	Hog sausage
Raw fermented sausages	Moist	Fermented	Fresh Thuringer
Raw fermented sausages	Moist	Fermented/smoked	Lebanon Bologna
Raw fermented sausages	Moist	Fermented	Merguez-type
Semi dry sausages	Semidried	Fermented, smoked	Summer sausage
Semi dry sausages	Semidried	Fermented, smoked	Thuringer
Semi dry sausages	Semidried	Fermented/short drying	Saucisson d'Alsace
Semi dry sausages	Semidried	Fermented/short drying	Laap Ch'eung
Semi dry sausages	Semidried	Fermented/short drying	Xun Chang
Semi dry sausages	Semidried	Fermented/short drying	Longaniza
Dry sausages	Dried	Fermented, long drying/ripening	Salami
Dry sausages	Dried	Fermented, long drying/ripening	Salchichón/saucisson
Dry sausages	Dried	Fermented, long drying/ripening	Chorizo
Dry sausages	Dried	Fermented, long drying/ripening	Pepperoni
Dry sausages	Dried	Fermented, long drying/ripening	Fjellmorr gilde
Dry sausages	Dried	Fermented, long drying/ripening	Turkish-style saudjouk
Cooked sausages	Moist	Cooking	Bologna
Cooked sausages	Moist	Cooking	Hot dogs
Cooked sausages	Moist	Cooking	Frankfurters
Cooked sausages	Moist	Cooking	Wieners
Cooked sausages	Moist	Cooking	Knockwurst
Cooked sausages	Moist	Cooking	Paté
Cooked sausages	Moist	Cooking	Bratwurst
Cooked sausages	Moist	Cooking	Mortadela
Cooked sausages	Moist	Cooking	Cotto salami

African countries and Southern European countries and can be briefly dried after fermentation. Raw fermented sausages can be smoked (e.g., Lebanon Bologna) or cooked (Table 59.1). They are preserved under refrigeration and are cooked before eating.

59.3.3 Semidry Sausages

There are many varieties of semidry sausages. As their denomination indicates, their moisture content is intermediate, between 35 and 50%, as a result of a mild drying and short ripening step. The water activity is above 0.86 and pH near 5.0. These sausages are fermented rapidly for a few hours in the United States or more slowly in Europe. They are smoked and can be cooked for pasteurization. The summer sausage is quite popular in the United States. Longaniza is typical in Spain and France. Semidry sausages are preserved under refrigeration unless the combination of low pH (below 5.0), and a moisture to protein ratio of less than 3.1:1 are achieved to guarantee the shelf-life (Sebranek 2004).

59.3.4 Dry Sausages

There are many dry types of sausage. In general, they have a moisture content below 35%. The denominations may vary according to the geographical area where they are produced. In other cases, the names may be applied depending on the physical or chemical

Figure 59.2 *Typical view of sliced salchichón.*

characteristics of the sausages like form, diameter, size of fat particles, application of smoking, addition of specific spices, presence of molds on the external surface, and so on. Examples of typical dry sausages are compiled in Table 59.1. Some of the most popular are salamis, which may have different diameters. There are salamis of 55–60 mm diameter and a ripening time of 28–32 days, like French Ménage, 14–21 days of ripening like the Italian Turista, or 14–18 days of ripening as for the Spanish Salchichón. A typical view of sliced salchichón is shown in Figure 59.2. Other salamis may have larger diameters such as 90–100 mm. In this case, ripening times are longer, up to 60–70 days as in the French Varzi and Italian Crespone Milano (Baldini and others 2000). Milanese salami is seasoned with garlic, white wine, peppercorns and sugar. Typical Mediterranean sausages are French saucisson, Spanish chorizo (Fig. 59.3) or Italian salami, while typical North-European products are German- or Hungarian-style salamis. Pepperoni and summer sausages constitute typical sausages in the USA (Toldrá 2004a).

Figure 59.3 *Slices of typical Spanish chorizo.*

59.3.4 Cooked Sausages

There are also many types of cooked sausages, including garlic sausages, frankfurters, mortadella, and so on. Italian mortadella, or Bologna sausage, is fermented at 30–38°C for 2–7 days. Then, it is cooked until it reaches an internal temperature of 65–70°C, cooled, and dried for maturation. German Bruhwurst is smoked and scalded. Kochwurst is also scalded and may contain large chunks of meat; it is eaten either sliced or spread (Fisher and Palmer 1995). Frankfurters, wieners, and hot dogs are coarsely minced or finely chopped (Knipe 2004). Cotto salami is cooked, contains peppercorns, and may be smoked. This type of cooked salami is refrigerated before eating.

59.4 INGREDIENTS AND ADDITIVES

59.4.1 Meat and Fat

Pork meat, either alone or in combination with beef, is the main ingredient of sausages. In other products like poultry sausages, the main ingredient is poultry. It is important to control the characteristics of all these raw meats, in particular hygienic quality (Roca and Incze 1990). Beef meat contributes to a better red color of the product. The same applies to the use of meat from older animals, which are richer in myoglobin (Toldrá 2006a).

The other main ingredient is fat which must be fresh or stored under frozen storage. It is convenient to avoid long frozen storages because endogenous lipases are active at low temperatures and can generate free fatty acids that are prone to oxidation and development of rancidity (Toldrá 2006b). Firm pork back fats with high melting point are the most adequate for sausage production (Lücke 1985). Soft fats can produce smearing during chopping. Fat unsaturation control through the iodine index and the amount of free fatty acids through the acid index (as an indicator of freshness) are recommended.

59.4.2 Salt

Salt is an essential ingredient of sausages, usually added at 2–3%. It contributes to the technological and sensory quality through

1. Selection of the microbial flora by inhibition of the growth of undesirable microorganisms;
2. A characteristic salty taste of the sausage;
3. An increase of myofibrillar protein (actin and myosin) solubility; and
4. Control of enzyme activity (Toldrá 2004b).

59.4.3 Nitrite and Nitrate

Nitrite constitutes an essential additive for sausage preservation. Sodium nitrite is formulated in a curing salt. In some cases, potassium nitrate can also be added in the formulation when sausages are ripened for a long time. In this case, nitrate acts as a reservoir of nitrite. Nitrate is reduced to nitrite by nitrate reductase activity from bacteria either naturally present in the mix or added as starter cultures (such as *Micrococcaceae*). Nitrite is very reactive and interacts rapidly with proteins, such as myoglobin, and inhibits the growth of

undesirable microorganisms, in particular *Clostridium botulinum* (Cassens 1997). Despite its effectivity against pathogens, the amounts of nitrate and/or nitrite added to the initial mixture are kept at a low level to keep residues as low as possible in the finished product. The reason for this is that nitrites can react with secondary amines, producing nitrosamines, which can exert toxic (i.e., cancerigen) effects (Cassens 1995). Ascorbic and erythorbic acids or their sodium salts are used to accelerate the reduction of nitrite to nitric oxide, assuring the absence of residual nitrite in the products and thus avoiding the risk of nitrosamine formation.

59.4.4 Carbohydrates

Carbohydrates are added as the substrate for microbial fermentation. Lactic acid is the main compound resulting from fermentation by lactic acid bacteria. Depending on the type and amount of carbohydrates, more or less lactic acid will be generated and pH will drop accordingly (Lücke 1985). Glucose or saccharose favor a faster pH drop. If an excessive amount of carbohydrates is added, pH can drop to values below 4.7 and most of the enzymes responsible for the generation of flavor compounds can be inhibited and the sensory quality seriously affected. However, the addition of low amounts or long-chain carbohydrates may result in a deficient pH reduction that will allow the growth of undesirable microorganisms (Toldrá 2006c).

59.4.5 Spices and Flavorings

There is a wide variety of spices and flavorings that can be used for seasoning the sausages. Spices can also contribute to a specific flavor or color. There is a great variety of seasoning formulations for different types of sausages (Coggins 2001). This includes mustard, oregano, rosemary, garlic, onion, pepper, or paprika. Spices can be used either in a natural form (whole or ground) or as flavoring extracts (essential oils and oleoresins). Flavoring agents and flavor enhancers may be used to accentuate a specific flavor. Smoke flavoring may be applied on the surface as an oil or water solution to give a smoke flavor (Ellis 2001).

59.4.6 Starter Cultures

The use of starter cultures for sausage production has expanded in recent years. The use and properties of starter cultures are fully described in the chapter on fermented meat production.

59.5 PROCESSING TECHNOLOGY

There are some essential stages in the processing of sausages. Outlines of the main unit operations in the processing of semidry and dry sausages, cooked sausages, and emulsified sausages are shown in Figures 59.4 and 59.5, respectively. Several operations are common for the different processes but others are specific for each type of product. There are some differences as follows:

1. Dry and semidry sausages are stuffed into porous casings that allow water evaporation, and cooked sausages are stuffed into nonpermeable casings to guarantee water retention during cooking;

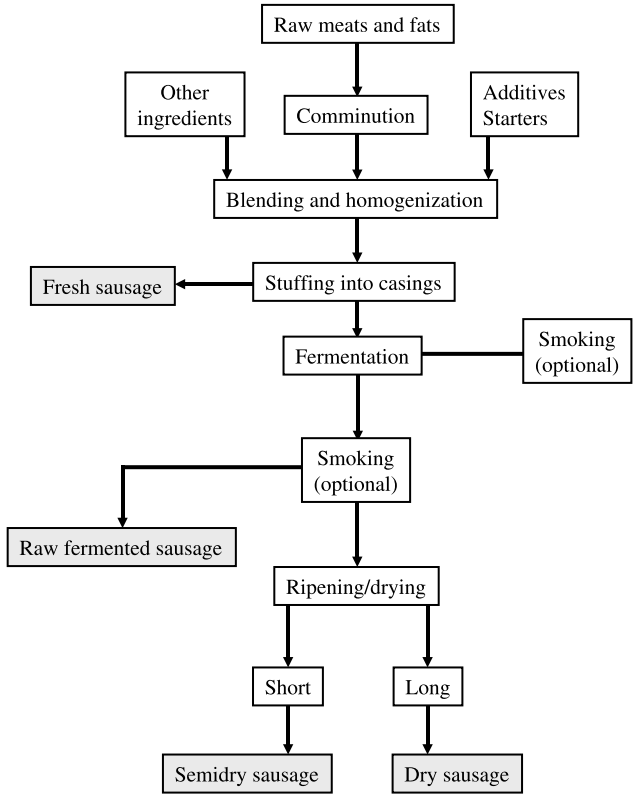

Figure 59.4 *Process flow diagram for the processing of fresh raw fermented, semidry and dry sausages.*

2. Dry and semidry sausages are usually fermented and afterwards submitted to ripening and drying, but cooked sausages are cooked just after stuffing and chilled;
3. The shelf-lives of these types of products are also different because dry sausages are stable at room temperature, but the others must be preserved in cold temperatures. The basic operations are described in the following.

59.5.1 Comminution and Stuffing

Raw meats and fats are chopped and comminuted in a grinder under cold temperatures. The size of lean and fat particles will depend on the plate size during grinding (Roca and Incze 1990). The pieces of a grinder are shown in Figure 59.6. The rest of the ingredients and additives, including microbial starters, are added to the resulting ground mass. Homogenization of the ground mass is a very important step in obtaining a good distribution of the additives through the mass. The batter is homogenized under cold temperatures by mixing under vacuum for removing as much oxygen as possible. An example of the homogenized batter is shown in Figure 59.7. In the case of cooked sausages, the comminution is performed in a cutter machine. The batter is stuffed into natural (mostly for traditional products) or man-made (collagen or synthetic) casings by using vacuum filling machines. In the case of dry or semidry sausages, casings must be porous

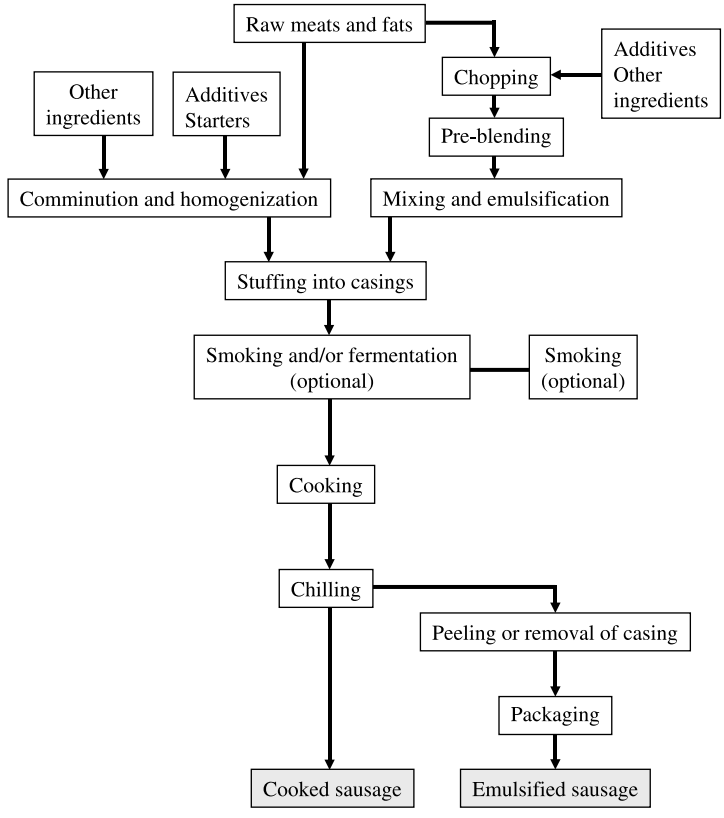

Figure 59.5 *Process flow diagram for the processing of cooked sausages.*

Figure 59.6 *Main process of a grinder.*

Figure 59.7 *View of the batter after homogenization.*

and the pore size of the casings must allow evaporation of water during fermentation/ripening/drying (Acton 1977). In the case of cooked sausages, casings made with co-extruded collagen, alginate, or plastic are usual (Rust 2004).

59.5.2 Emulsification

In the case of emulsified cooked sausages, emulsification procedures must be followed. The mixer–emulsifier process consists of a mixing step and an emulsifying step. Preblended lean meat and fat mixtures including spices, binders, and so on, are combined in a mixer and the mixing continues until the temperature reaches 5–7°C, which is below the emulsion temperature that depends on the predominant source of fat. The mixture is then passed through the emulsifier several times depending on the temperature rise and is stuffed into the casing (Knipe 2004).

59.5.3 Fermentation

This stage is fully described in the chapter on fermented meat production. The main objective is to activate microorganisms, added in the starter cultures, and achieve a good microbial development. Fermentation temperatures are lower in Europe, around 22–26°C, for *L. sakei* or *L. plantarum* to produce lactic acid and reach the desired pH drop. Fermentation temperatures are higher in the United States, around 30–37°C, to allow typical starters like *L. plantarum* or *P. acidilactici* to produce high amounts of lactic acid and achieve pH values below 5.0 to 4.6. Rapid acidification ensures the inhibition of spoilage microorganisms (Toldrá 2006d).

59.5.4 Smoking

Smoking constitutes a traditional technology, especially in Northern countries, used for the preservation of food stuffs. Its application depends on the type of product and the

country. The smoke flavoring can be sprayed or atomized on the surface of the sausage. This flavoring is a liquid produced by distillation and subsequent condensation of volatile compounds from smoke (Ellis 2001). It can be applied at different stages of the processes: during fermentation, ripening, or even at the end of the process. In the case of cooked sausages, smoking may be applied during cooking or afterwards.

Smoking imparts a characteristic flavor and gives a darker external color to the product. It also exerts antioxidant properties due to the phenolic portion of the smoke and contributes to preservation due to the antimicrobial properties of smoke. However, some undesirable effects of smoking are related to formaldehyde and other possible carcinogenic substances contained in the smoke process (Bem and others 1995).

59.5.5 Ripening/Drying

This stage is applied to semidry and dry fermented sausages. Its main objective is to reduce water content and favor the development of characteristic flavor. Sausages are hung in racks and placed in drying chambers. The conditions for ripening and drying (time, temperature, and relative humidity) depend on the size and type of product. Temperature is milder for long processing times and higher for short processes. Air velocity is fixed at mild speed, around 0.05–0.1 m/s, and helps to homogenize the humidity in the chamber environment. Relative humidity is decreased progressively to avoid excessive dehydration of the sausages. Drying is a delicate stage where water must diffuse from inside the sausage to the outer surface and then is evaporated from the outer surface to the environment. However, both processes, diffusion and evaporation, must proceed at similar rates to have the correct drying. If evaporation is too intense and rapid, sausages may develop case hardening, an usual defect consisting in an excessively dry and hard outer layer (Toldrá 2002).

59.5.6 Cooking

Cooking ensures microbial destruction of microorganisms and inactivation of a good number of enzymes. It also helps to develop characteristic color and flavor of the product. Cooking is carried out in forced convection ovens either in batch or continuous operation. Smoking may be simultaneously applied during heating. The heat treatment is calculated to inactivate pathogen and spoilage microorganisms for extended shelf-life of the product with minor effect on sensory characteristics (Guerrero-Legarreta 2006). Cooking can be considered as pasteurization because the temperature in the sausages reaches around 72°C for 30–60 min. Chilling is also a delicate stage to ensure the wholesomeness of the sausages and complete food safety.

59.5.7 Packaging

Typical consumption of sausages occurs once they are sliced. Sliced sausage constitutes a common commercial presentation for its sale in supermarkets. However once the sausage is sliced, it is not protected by the casing and is thus exposed to rehydration or dehydration, oxidation, recontamination, and so on. The basic requirements for these packages, in addition to extreme hygienic conditions for packaging, are a high water vapor barrier, high barrier to oxygen, and reduced headspace or headspace with low oxygen levels (Toldrá and others 2004). Vacuum packaging and modified atmosphere packaging

(MAP) have been widely used for the preservation of sliced sausages. Both systems are effective for preserving color, suppressing microbial spoilage, and extending shelf-life. However, slices are firmly adhered in the vacuum packaging and the unpleasant separation of slices can be avoided with MAP (Sebranek and Houser 2006). More recently, active and intelligent packagings are being developed to retain the maximum quality of the product.

59.6 SENSORY CHARACTERISTICS

The color of sausages mainly depends on the concentration of its natural pigment myoglobin. Its content depends on the type of muscle and the age of the animal, being higher in older animals and in muscles with oxidative pattern (Aristoy and Toldrá 1998). The typical color of semidry and dry sausages is due to the formation of nitrosylmyoglobin by reaction of nitric oxide, generated from nitrite, with myoglobin. In the case of cooked sausages, the color changes from red to pink during the heating process, especially at temperatures above 65°C. This is due to the transformation of nitrosylmyoglobin into nitrosylhemochrome, which has a typical light pink cured-meat color. Upon heating, globin denatures and detaches itself from the iron atom, and surrounds the heme moiety. This color is also known as cooked cured-meat pigment (Pegg and Shahidi 2000).

The texture of semidry and dry sausages depends on the degree of drying and pH drop as low pH coagulates proteins and makes the texture harder. The content of fat and its composition also affects texture. In the case of cooked sausages, texture depends on several factors, such as the extent of heating (structure breakdown), the moisture content, the extent of proteolysis (degree of myofibrillar protein breakdown), and the content of connective tissue. The content of intramuscular fat also exerts a positive influence on some texture and appearance traits. Starches and vegetable proteins added to the formulation help to enhance texture and bind water as well as improve the mouthfeel.

As in the case of ham, proteolysis and lipolysis contribute to the generation of free amino acids, and fatty acids, which will contribute to the generation of taste and aroma. The flavor of sausages is described in the chapter on processed pork meat flavors.

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Fermented Meat Production

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60.1 Introduction 266
60.2 Microbiology of Meat Fermentation 266
60.2.1 Lactic Acid Bacteria (LAB) 266
60.2.2 Micrococceae 266
60.2.3 Yeasts 267
60.2.4 Molds 268
60.3 Microbial Starters 268
60.4 The Microbial Proteolytical System 269
60.4.1 Endo-Peptidases 269
60.4.2 Exo-Peptidases 269
60.4.3 Transformation of Amino Acids 270
60.5 Biochemical Changes During Fermented Meat Production 271
60.5.1 Glycolysis in Fermented Meat 271
60.5.2 Proteolysis in Fermented Meat 271
60.5.3 Lipolysis in Fermented Meat 272
60.5.4 Oxidation in Fermented Meat 273
60.5.5 Generation of Undesirable Substances 273
60.6 Trends for Improved Fermented Meat 274
60.6.1 Enzyme Addition for the Improvement of Sensory Quality 274
60.6.2 Probiotic Addition for the Improvement of Nutritional Quality 274
References 275

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60.1 INTRODUCTION

The consumption of fermented food products is one of the oldest known practices. Traditionally, meat fermentation was based on the development of desirable indigenous flora. However, it is evident that not all species constituing the microbial flora present in the product contribute desirably to fermentation. So, there is a positive flora contributing to the specific characteristics of the meat product and a negative flora that can inhibit the growth and/or development of the positive flora and, what is worse, produce undesirable transformations of the substrate, resulting in important sensory and safety problems. Thus, the composition of the microbial flora may change at the site of production depending on many parameters and, if the positive flora is substantially decreased, the fermented meat products may lack adequate organoleptic characteristics. The need for a consistent quality and regular production suggests a need for flora reinforcement. In some cases, natural flora has been reinforced by back-slopping which, consists of the addition of small amounts of previously fermented meat having good sensory properties (Toldrá 2002). This practice was used for decades but gave heterogeneous quality.

Microbial starters, initiated in the United States in the 1950s and in Europe in the 1960s, revolutionized the processing of fermented foods in general and also in fermented meats. Today, fermented meats are generally produced with microbial starters. The microbiology of meat fermentation and the use of microbial starters are described in this chapter.

60.2 MICROBIOLOGY OF MEAT FERMENTATION

For many decades, meat fermentation was based on the development of the indigenous flora, in particular the positive flora. This flora has been studied and, more recently, these microorganisms have been isolated and biochemically identified through molecular methods applied to extracted DNA and RNA (Rantsiou and Cocolin 2006). Examples of bacteria identified through these modern methods are shown in Table 60.1, including a general description of the main groups of natural flora typical of fermented meat.

60.2.1 Lactic Acid Bacteria (LAB)

These bacteria metabolize glucose or other carbohydrates through either homo or hetero-fermentative pathways and generate lactic acid. This acid is accumulated into the product and drops the pH towards acid values. In some cases, where there is a heterofermentative pathway, some undesirable secondary products such as acetic acid, hydrogen peroxide, acetoin, and so on may be generated. Growing temperatures differ depending on the species. So, *L. sakei* and *L. curvatus* grow at mild temperatures, and are mainly used in European sausages where mild fermentation temperatures are typically used. On the other hand, *L. plantarum* and *P. acidilactici* grow well at higher temperatures (30–35°C) and are used in the United States where fermentation temperature is higher (Toldrá 2006a). Lactic acid bacteria have a proteolytic system (described later), and are also able to generate different types of bacteriocins with antimicrobial properties.

60.2.2 Micrococceae

This group of microorganisms is mainly composed of *Staphylococcus* and *Kocuria* (formerly *Micrococcus*). These microorganisms have proteolytic and lipolytic activity,

TABLE 60.1 Examples of the Main Microorganisms Isolated from Different Typical and Naturally Fermented Meat Products.

Microorganism	Product	Country	Reference
L. sakei	Salame friulano	Italy	Rantsiou and others (2005)
	Soppressata	Italy	Andrigetto and others (2001)
	Fuet	Spain	Aymerich and others (2003)
	Chorizo	Spain	Aymerich and others (2003)
L. curvatus	Salame friulano	Italy	Cocolin and others (2000)
	Sausage	Hungary	Rantsiou and others (2005)
	Fuet	Spain	Aymerich and others (2003)
	Chorizo	Spain	Aymerich and others (2003)
L. plantarum	Sausage	Greece	Rantsiou and others (2005)
	Salame friulano	Italy	Comi and others (2005)
	Salame tradizionale	Italy	Andrigetto and others (2001)
	Fuet	Spain	Aymerich and others (2003)
	Chorizo	Spain	Aymerich and others (2003)
L. curvatus *S. xylosus*	Salame friulano	Italy	Rantsiou and others (2004)
	Salsiccia	Italy	Rossi and others (2001)
	Fuet	Spain	Aymerich and others (2003)
	Chorizo	Spain	Aymerich and others (2003)
K. varians	Salame friulano	Italy	Cocolin and others (2001a)
S. carnosus	Salame friulano	Italy	Cocolin and others (2001b)

and contribute to flavor and to nitrate reductase activity, which reduces nitrate to nitrite and contributes to the typical color formation and safety. These microorganisms grow little at the onset of fermentation unless they are already present in high amounts. The species from this family also have catalase activity, which helps to prevent lipid oxidation and contributes to color stability.

60.2.3 Yeasts

Debaryomyces hansenii is the predominat yeast in fermented meats. This yeast grows preferentially in the outer area of the sausage due to its aerobic metabolism. *D. hansenii* has a good lipolytic activity and is able to degrade lactic acid. In addition, it contributes to the generation of volatile compounds from branched-chain amino acids (Durá and others

2004a). This yeast has an important deaminase/deamidase activity able to act on certain free amino acids and generate ammonia as a byproduct, which raises the pH in the sausage (Durá and others 2002).

60.2.4 Molds

The presence of molds on the outer surface of sausages is typical in certain areas, for example in Mediterranean dry fermented sausages. These molds give a characteristic white coating on the surface. The most usual molds identified are *Penicillium nalgiovense* and *P. chrysogenum*, which grow in the sausages from the indigenous flora, or houseflora, of the processing plant (Sunesen and Stahnke 2003). These molds contribute to flavor through their proteolytic and lipolytic activity and can also generate ammonia through deaminase and deamidase activity.

60.3 MICROBIAL STARTERS

Microorganisms used as starter cultures must have good stability under the processing conditions. This means that they must resist acid pH and tolerate the presence of salt and low water activity. These microorganisms must also be able to grow at fermentation temperatures (i.e., 18–25°C in Europe or 35–40°C in the United States). They must also have a good enzyme profile for the generation of the desired products (lactic acid for pH drop, volatile compounds for aroma, nitrate reduction, secretion of bacteriocins, and so on) and a lack of undesirable enzymes like decarboxylases that generate amines. Thus, the selection of strains is extremely delicate. The most important microorganisms used as starters belong to the lactic acid group of bacteria, *Micrococacceae*, yeasts, and molds (Leistner 1992). More specifically, the most important are *Lactobacillus sakei*, *L. curvatus*, *L. carnosus*, *L. plantarum*, *P. pentosaceus*, *Lactococcus lactis*, *Kocuria varians*, *Staphylococcus xylosus*, the yeast *Debaryomices hansenii*, and the molds *Penicillium*

TABLE 60.2 Main Enzyme Activities of Microorganisms and Their Effects in Fermented Meats.

Microorganisms	Enzyme Activity	Effects
Lactic acid bacteria	Glucohydrolase	Lactic acid generation
	Endoprotease	Protein breakdown
	Exoprotease	Generation of free amino acids
Micrococacceae	Nitrate reductase	Nitrate reduction to nitrite
	Lipase	Generation of free fatty acids
	Exoprotease	Generation of free amino acids
	Catalase	Degradation of hydrogen peroxide
Yeasts	Lipase	Generation of free fatty acids
	Transaminase	Transformation of amino acids
	Deaminase/deamidase	Lactic acid consumption and generation of ammonia
Molds	Lipase	Generation of free fatty acids, ready for oxidation
	Exoprotease	Generation of free amino acids
	Transaminase	Transformation of amino acids
	Deaminase/deamidase	Generation of ammonia

nalgiovense, and *P. chrysogenum* (Toldrá 2004, 2006b). The main enzymes and their effects for each group of microorganisms are shown in Table 60.2. The use of bacterial starter cultures with protective effects, also known as protective cultures, has the main advantages of safety enhancement and prevention of potential food-borne microbial hazard (Hugas and others 2002; Talon and Leroy 2006).

60.4 THE MICROBIAL PROTEOLYTICAL SYSTEM

The proteolytic system in microorganisms is essential for a large number of cellular processes involved in the use of proteins to supply free amino acids as nutrients and gene regulation (Pritchard and Coolbear 1993). This system is composed of endo-peptidases or proteinases, which are predominantly extracellular and bound either to the cell wall or to the cell membrane (Visser 1993), and peptidases, which are located inside the cell. The mode of action can vary if using cell-free extracts where all the enzymes will be in contact with the substrates. However, when using whole cells, which is the normal situation, the amino acid and peptide transport system will regulate the supply of the amino acids required for growth (Tan and others 1993). The cell wall proteinase is the first enzyme to degrade proteins approaching the cell. Once the generated large peptides are transported into the cell, they are further degraded to small peptides and free amino acids by different peptidases and amino-peptidases (Bockelman 1995).

60.4.1 Endo-Peptidases

The proteolytic activity of several lactic acid bacteria like *L. sakei*, *L. curvatus*, *L. plantarum*, and *L. carnosus* has been assayed, as either whole cells or cell-free extracts, against meat proteins (Fadda and others 1999a,b; Sanz and others 1999a,b). Lactic acid bacteria have some proteinases associated with the cell membrane that are responsible for initial protein breakdown. These proteinases have shown poor effectivity in hydrolyzing myofibrillar proteins, but are able to hydrolyze sarcoplasmic proteins, especially *L. plantarum* and *L. casei* (Sanz and others 1999a). The peptide profiles resulting from these hydrolyses have also been studied. A large number of hydrophilic peptides, correlated to desirable cured-meat flavors, have been detected. Other hydrophobic peptides, correlated to bitterness, have also been detected (Aristoy and Toldrá 1995; Henriksen and Stahnke 1997). Yeasts have also shown proteolytic activity. *D. hansenii* has a protease B that is active at neutral–basic pH (Bolumar and others 2005, 2006). This protease has been able to hydrolyze sarcoplasmic proteins. This activity has been observed when using either whole cells or cell-free extracts (Santos and others 2001). However, this enzyme is inactivated at acid pH, reducing its expectatives in fermentation of pH levels of 5.0 or even lower.

60.4.2 Exo-Peptidases

Lactic acid bacteria contain several important exo-peptidases that contribute to the generation of small peptides and free amino acids during fermentation. These enzymes are located inside the cell so that cell-free extracts from *L. sakei*, *L. curvatus*, and *L. casei* of myofibrillar and sarcoplasmic proteins give a net increase in the content of free amino acids. These extracts give particularly significant increases in glutamic acid, alanine, and leucine for *L. sakei*, glutamic acid and alanine for *L. curvatus*, and arginine

and glutamic acid for *L. casei* (Fadda and others 1999a; Sanz and others 1999a,b). In the case of *L. plantarum*, there was a decrease, probably due to lower exo-peptidase activity or higher intracellular metabolic activity for amino acid degradation (Fadda and others 1999b). There are a wide variety of exo-peptidases in lactic acid bacteria. For instance, the following exo-peptidases have been purified and characterized from a typical meat lactobacillus like *Lactobacillus sakei*:

- A general aminopeptidase, with optimal neutral pH, and a broad range of activity against amino acids, especially alanine and leucine (Sanz and Toldrá 1997);
- An arginine aminopeptidase, with optimal acid pH, and preference for basic residues such as arginine and lysine (Sanz and Toldrá 2002);
- A dipeptidase with optimal basic pH and a broad specificity against dipeptides except those containing Pro or Gly at the N-terminus (Montel and others 1995);
- A tripeptidase with optimal neutral pH and able to hydrolyze a wide spectrum of tri-peptides except those with Pro in the second position (Sanz and others 1998); and
- A X-prolyl-dipeptidylpeptidase, with optimal neutral pH and able to hydrolyze X-Pro dipeptides from the amino terminus from different peptides (Sanz and Toldrá 2001).

The yeasts (*Debaryomices hansenii*) also have several exo-peptidases such as a prolyl aminopeptidase, with an optimal neutral pH, and restricted to the hydrolysis of Pro at the amino terminus of peptides (Bolumar and others 2003a), and an arginyl aminopeptidase, with an optimal neutral pH and maximum specificity for basic residues like arginine and lysine (Bolumar and others 2003b). Incubations with whole cells and cell-free extracts of *Debaryomices hansenii* have shown a good hydrolysis rate of sarcoplasmic proteins with the generation of hydrophilic and hydrophobic peptides and free amino acids (Santos and others 2001).

60.4.3 Transformation of Amino Acids

Microorganisms have further enzymes (decarboxylases, deaminases, and so on) able to transform amino acids and affect the sensory characteristics of the final product (Ordoñez and others 1999; Toldrá and others 2001). These enzymes use free amino acids, generated from proteolysis, as substrate. Decarboxylation of amino acids may produce biogenic amines from respective amino acids (see Table 60.3). Transamination

TABLE 60.3 List of Amines (and Their Origins) that can be Generated in Fermented Meats.

Compounds	Origin
Tyramine	Tyrosine
Tryptamine	Trytophane
Phenylethylamine	Phenylalanine
Cadaverine	Lysine
Histamine	Histidine
Putrescine	Ornithine
Spermine/spermidine	Methionine

consists in the transference of the α-amino group of the first amino acid to the α carbon atom from an α-keto acid, generating a keto acid from the first amino acid and a new amino acid. Dehydrogenases transform the amino acid in the corresponding keto acid and ammonia. Deamidation and deamination also generates ammonia (Durá and others 2002). The microbial degradation of the amino acid side chain of tyrosine and tryptophan by liases may lead to phenol and indole formation, respectively (Molinard and Spinnler 1996). The Strecker degradation of amino acids produces branched aldehydes, like 3-methylbutanal, 2-methylbutanal and phenylacetaldehyde from leucine, isoleucine, and phenylalanine, respectively (Ordoñez and others 1999). Strecker degradation can produce sulfur compounds with high negative aroma impact when affecting sulfur-containing amino acids like methionine, cysteine, and cystine, which have low threshold values and a high aromatic impact (Flores and others 1998).

60.5 BIOCHEMICAL CHANGES DURING FERMENTED MEAT PRODUCTION

60.5.1 Glycolysis in Fermented Meat

Lactic acid is the main product resulting from carbohydrate fermentation. The generation rate and the final amount will depend on the type of lactic acid bacteria used as starter, the type and amount of added carbohydrates (glucose is easily fermented), the fermentation temperature (higher in the United States and lower in Europe), and other processing parameters (such as amount of salt and time of ripening). Added carbohydrates (glucose, sucrose, and so on) are transported into the cell and start the glycolytic or Embden–Meyerhof pathway. Key enzymes in this pathway are aldolases, which generates glyceraldehyde-3-phosphate, pyruvate kinase, which generates pyruvate from phosphoethanol pyruvate, and lactate dehydrogenase, which generates lactic acid from pyruvate (Demeyer 1992). When the pathway is heterofermentative, some additional end products such as acetate, formate, ethanol, and acetoin may be generated in trace amounts and can affect the sensory quality of the sausage (Demeyer and Stahnke 2002). The generated lactic acid may be either the D(−) or L(+) configuration, or a mixture of both, depending on the species of lactic acid bacteria used as starter. The ratio between the L and D enantiomers will depend on the actions of L and D lactate dehydrogenase, respectively, and the presence of lactate racemase (Demeyer and Toldrá 2004).

The generation of lactic acid is important because it lowers the pH towards acid values. Acid pH favors protein coagulation as it approaches the isoelectric point of myofibrillar proteins, and thus also favors water release (Toldrá 2004). Furthermore, safety is improved because of the inhibition of undesirable, pathogen, or spoilage bacteria. The pH drop favors initial proteolysis (by stimulating the activity of muscle cathepsin D) and lipolysis (by stimulating lysosomal acid lipase), both active at acid pH. However, excessive acidity may inhibit other enzymatic reactions related to the generation of flavor compounds (Toldrá and Verplaetse 1995).

60.5.2 Proteolysis in Fermented Meat

Muscle and microbial proteases have a combined action during meat fermentation. The relative contribution of each group of enzymes depends on the type of meat and the microorganisms used as starter cultures. The enzyme activity also depends on the applied

temperature and extent of the process. To evaluate these contributions, some studies have been carried out using antibiotics and other protease inhibitors. Muscle cathepsin D, which is very active at pH 4.5, appears to be the protease that initiates the degradation of myosin and actin, and other muscle proteases, like cathepsins B and L, are limited to a few proteins like actin and its degradation products (Molly and others 1997). Myofibrillar proteins, in particular myosin, α-actinin, and actin, have been reported to be degraded (García and Fox 1991). Lactobacilli have shown a good ability to degrade sarcoplasmic proteins (Fadda and others 1999a,b; Sanz and others 1999a,b).

Once proteins are broken down, muscle and microbial peptidases generate a good number of peptides that are further degraded to small peptides and amino acids, but there are large variations in the amounts generated. The highest nonprotein nitrogen value has been reported in sausages with pH value below 4.7 (Flores and others 1997). Some of these peptides have been sequenced and the parent protein identified by comparison of homologies. So, five peptides originating from myoglobin, creatin kinase, troponin T, troponin I, and myosin light chain 2, have been derived (Hughes and others 2002). Muscle and microbial tri- and dipeptidylpeptidases are responsible for the generation of small tri- and dipeptides, but their activity depends on the pH and the level of salt that inhibits this type of enzymes (Sentandreu and Toldrá 2001). The generation of small peptides may be depressed by the level of salt that inhibits muscle peptidases (Sanz and Toldrá 1999; Sanz and others 2002; Toldrá 2004), although intense levels of nonprotein nitrogen, up to 20% of the total nitrogen content, may be reached. This is the reason for wide variations in increases in nonprotein nitrogen, from 2- to 12-fold. The final step consists in the generation of free amino acids by aminopeptidases, especially muscle alanyl and methionyl aminopeptidases, together with major aminopeptidase from *L. sakei*. Basic amino acids, such as arginine and lysine, would be generated by muscle and microbial arginyl aminopeptidases (Toldrá 2006c). Some amino acids have been correlated with taste descriptors like spicy, beefy, sweet, bitter, and astringent (Talon and others 2004). Microbial metabolism of leucine, valine, and isoleucine may be responsible for the generation of branched-chain aldehydes and secondary products such as acids, alcohols, and esters (Toldrá 2006b). This metabolism is found in the *Micrococcaceae* family (Staphylococcus and Kocuria) and *Debaryomices hansenii*, and to a less extent in Lactobacilli (Demeyer and others 2000). The incubation of leucine with *Staphylococcus xylosus* and *S. carnosus* generates other volatile compounds such as 3-methylbutanoic acid and α-hydroxy isocaproic acid (Olesen and others 2004a), although curing salts have a pronounced effect on the level of volatile compounds (Olesen and others 2004b).

60.5.3 Lipolysis in Fermented Meat

Lipolysis consists of the generation of free fatty acids through the breakdown of triacylglycerols by lipases and phospholipids by phospholipases. The released fatty acids exert a direct effect on taste (sourness) and an indirect effect on aroma through generation of volatile compounds via oxidative reactions. Lipolysis in fermented meat is the result of the action of muscle and microbial enzymes (Hierro and others 1997). The percentage contribution of muscle lipolytic enzymes to total fat hydrolysis is estimated to be around 60–80%, the rest being due to microbial lipases (Molly and others 1997). Lysosomal acid lipase and acid phospholipase are the main muscle lipases, and the hormone-sensitive lipase and the monoacylglycerol lipase are the most important in adipose tissue (Toldrá 1992, 1998). These enzymes have shown good stability throughout the full process (Motilva and others 1992, 1993a,b), and their activity will vary depending

on the conditions found in the sausages (pH, salt concentration, temperature, and water activity) (Motilva and Toldrá 1993). Oleic, linoleic, estearic, and palmitic acids constitute the free fatty acids generated at a faster rate and most of them proceed from phospholipid degradation (Toldrá 2002). Rapid rates are also observed in adipose tissue fat, although they proceed from triacylglycerols. Those fatty acids with double bonds are prone to further oxidative reactions and generate volatile compounds (Buscailhon and others 1994). Starter cultures have a definitive effect on the formation of flavor in dry sausages (Berdagué and others 1993).

60.5.4 Oxidation in Fermented Meat

Fatty acids with double bonds (mono- and polyunsaturated fatty acids) are susceptible to further oxidative reactions. These reactions may generate volatile compounds with desirable aroma or some compounds with unpleasant aroma. Off-flavor development is typical of excessive oxidation (Skibsted and others 1998). Oxidation can be initiated by muscle oxidative enzymes, such as peroxydases and cyclooxygenases, which catalyze the formation of free radicals. Other catalyzers are external light, heating, and the presence of moisture and/or metallic cations (Toldrá and others 2001). Peroxide radicals propagate the reaction of free radicals with oxygen. Hydroxyperoxides (primary oxidation products) are formed, but are very reactive and generate secondary oxidation products that contribute to flavor. Numerous volatile compounds with aroma properties are generated by oxidation (Stahnke 2002). The main products from lipid oxidation are aliphatic hydrocarbons (poor contribution to flavor), alcohols (high odor threshold), aldehydes (low odor threshold), and ketones. Alcohols may interact with free carboxylic fatty acids, giving esters, especially when nitrate is not used (Toldrá 2006b).

60.5.5 Generation of Undesirable Substances

The generation of undesirable compounds depends on several factors, but the most important is the hygienic quality of the raw materials. The most important amines that can be generated during meat fermentation are listed in Table 60.3. Microorganisms with decarboxylase activity contribute to the generation of biogenic amines. For instance, certain lactic acid bacteria may produce tyramine through decarboxylation of tyrosine (Eerola and others 1996). Even though tyramine releases noradrenaline from the sympathetic nervous system and the peripheral vasoconstriction and increase in cardiac output result in higher blood pressure and risk for hypertensive crisis (Shalaby 1996), its tolerance level is relatively high, between 100 and 800 mg kg^{-1} (Nout 1994). Other microorganisms in the contaminating flora may be generating cadaverine and/or putrescine. Starter cultures decrease the levels of putrescine considerably (Kalac 2006). Phenylethylamine may cause migraine and increases in the blood pressure, and histamine may excite the smooth muscles of the uterus, the intestine, and the respiratory tract. The levels of amines in fermented sausages are very variable (Hernández-Jover and others 1997). This variability is a result of several factors, such as the quali–quantitative composition of microbial flora, the chemical–physical variables, the hygienic conditions of raw meats, as well as the procedure adopted during production and the availability of precursors (Suzzi and Gardini 2003). Therefore, it is necessary to control the hygienic conditions of the raw materials, use microorganisms lacking decarboxylase activity, and apply good manufacturing practices (Talon and others 2002).

Finally, the generation of nitrosamines in fermented meat is usually very low or even negligible because there is a limited amount of nitrite remaining in the product able to react and produce nitrosamines (Cassens 1997). Some oxides of cholesterol may be generated during meat fermentation. Oxides like 7-ketocholesterol and 5,6α-5,6-epoxycholesterol have been linked to cardiovascular-related diseases, but analysis in European sausages has revealed very low levels, less than 1.5 μg/g, for exerting any toxic effect (Demeyer and others 2000). Other studies in Brazilian salamis have not found cholesterol oxides (Baggio and Bragagnolo 2006).

60.6 TRENDS FOR IMPROVED FERMENTED MEAT

60.6.1 Enzyme Addition for the Improvement of Sensory Quality

Different enzymes have been added to fermented meat in order to accelerate the process and improve flavor development. Some examples include a lactobacillus serine proteinase (Naes and others 1995), a proteinase (alcalase) from *Bacillus licheniformis*, and a proteinase from *L. paracasei* subsp. *paracasei* (Hagen and others 1996), neutral proteinase (neutrase) from *Bacillus subtilis* (Zapelena and others 1998), neutrase, alcalase, HT proteolytic from *B. subtilis* var. *amiloliquefaciens* and fungal protease from *Aspergillus oryzae* (Zapelena and others 1997), and pronase E from *Streptomyces griseus* (Díaz and others 1993). Other strategies have comprised the addition of cell-free extracts from *D. hansenii* and *L. sakei* (Bolumar and others 2006c). Some have improved sensory scores and overall acceptability of the sausages. In other cases, like the addition of papain and bromelain, the result was an undesirable flavor and texture (Ansorena and others 2002). Another important inconvenience of proteinases is the risk of introducing excessive texture softening. Other assayed alternatives have consisted of the addition of whole cells or cell-free extracts in an attempt to accelerate and improve the sensory quality of the sausages (Durá and others 2004a–c).

60.6.2 Probiotic Addition for the Improvement of Nutritional Quality

The possibility of using probiotics in fermented meats has been studied recently. Strategies are based on using existing commercial starter cultures or studying the survival in a meat environment of lactic acid bacteria of intestinal origin. In all cases, these bacteria must grow to numbers that may have health-promoting benefits (Leroy and others 2006). Sensory and technological properties must not be negatively affected. Some bioprotective lactic acid bacteria, selected to produce bacteriocins and low-molecular-mass antimicrobial compounds, have been found useful in the small intestine against food pathogens (Työpponen and others 2003). Other authors have investigated potential probiotic properties of several Lactobacillus strains (Pennacchia and others 2006). These authors found that the analyzed strains had good adhesion capability to human intestinal Caco-2 cell lines and good growth behavior in the presence of prebiotic carbohydrates, but further *in vivo* assays are necessary to fully demonstrate the probiotic properties. This field will no doubt experience rapid development in the next few years.

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61

Processed Pork Meat Flavors

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61.1 Introduction 282
61.2 Enzymatic Generation of Taste Nonvolatile Compounds in Meat Products 282
61.2.1 Proteolysis 283
61.2.2 Lypolysis 283
61.2.3 Nucleotides Breakdown 284
61.2.4 Glycolysis 284
61.3 Taste Contribution of Nonvolatile Compounds 284
61.4 Mechanism of Generation of Volatile Compounds and Contribution to the Aroma of Meat Products 287
61.4.1 Carbohydrate Degradation 287
61.4.2 Amino Acid Degradation Reactions 288
61.4.3 Maillard Reactions 288
61.4.4 Strecker Degradation 289
61.4.5 Degradation of Ribonucleotides 289
61.4.6 Degradation of Thiamin 289
61.4.7 Lipid Degradation 289
61.5 Contribution of Spices to the Aroma of Meat Products 290
61.6 Contribution of Smoking to the Flavor of Smoked Meat Products 290
61.7 Identification and Relevance of Volatile Compounds to the Flavor of Meat Products 291
References 297

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61.1 INTRODUCTION

Most of the meat products produced today are based on traditional practices. These products are attractive to consumers because they offer a wide variety of colors, flavors, and textures. There are many factors affecting the sensory characteristics of meat products such as the meats used as raw materials (genetic type, feed, age, sex, and rearing system), microorganisms selected as microbial starters for the fermentation and type of processing technologies (cooking, drying, ripening, smoking, etc.). There are many different cured meat products although they could be grouped into two major groups: dry-curing and wet-curing. Manufacturing processes for the most representative products in both groups are briefly schematized in Figure 61.1. Dry-cured ham and dry-fermented sausages are the main representative of dry-curing. In these meat products a dry cure (salt plus nitrate and/or nitrite) without any added water is applied either on the surface of the ham or mixed with the mince for the sausage. In both cases, the products are dried and ripened for a relatively long period of time for dehydration and, simultaneously, for the enzymatic development of flavor (Toldrá 2002). Cooked ham and frankfurters are good representative of wet-curing. In these cases, as schematized in Figure 61.1, a pickle injection or brine soaking is used as vehicle for cure penetration into the product or, in the case of sausages, by mixing with the mince (Flores and Toldrá 1993). These products are generally cooked, optionally smoked, and are shorter in processing time. Flavor may be also modulated through the use of spices and/or condiments. All these types of foods will be considered in this chapter, with special attention to taste and aroma compounds.

61.2 ENZYMATIC GENERATION OF TASTE NONVOLATILE COMPOUNDS IN MEAT PRODUCTS

A great research effort on the biochemical changes and enzymes involved during meat ageing were performed during the 1970s and 1980s. However, scarce information on

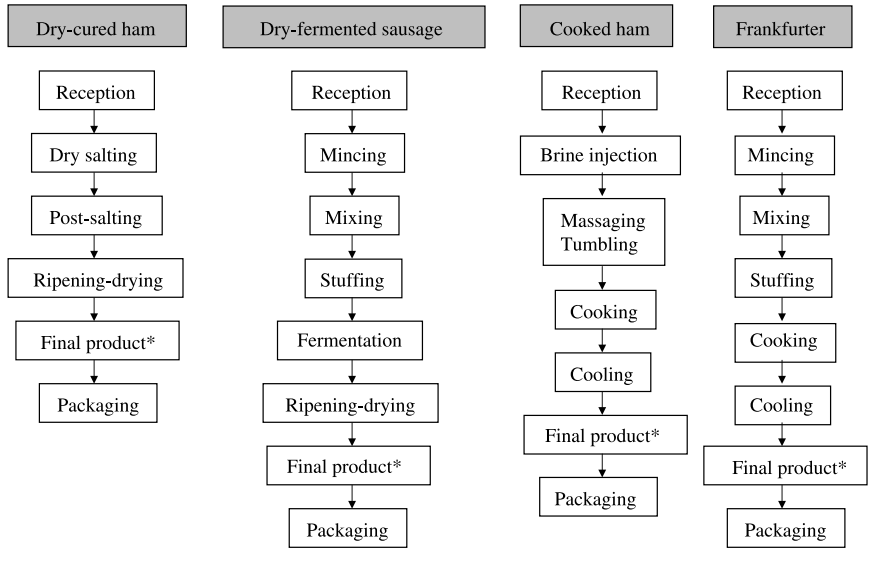

Figure 61.1 *Brief scheme of the manufacturing processes for representative products in dry-curing (dry-cured ham and dry-fermented sausage) and wet-curing (cooked ham and frankfurter). * The product may be optionally smoked.*

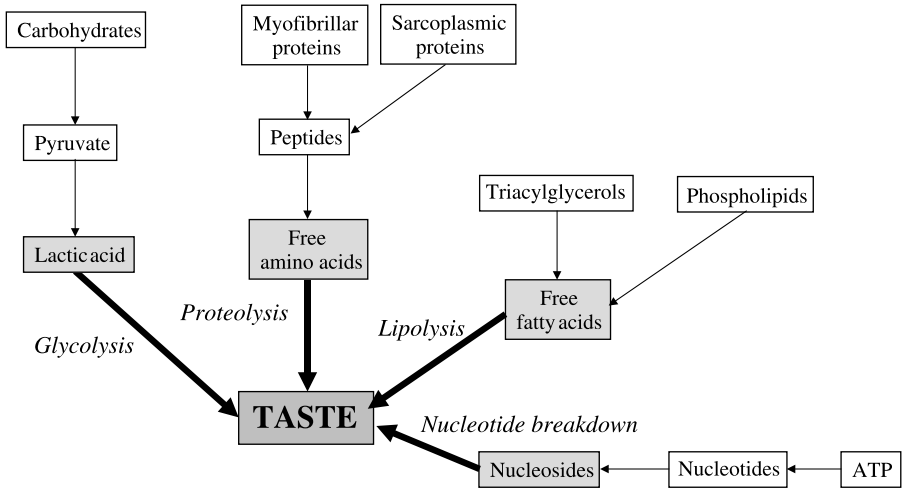

Figure 61.2 *Scheme of main biochemical mechanisms involved in taste development.*

biochemical changes was available in other products like cooked, dry-fermented and dry-cured meats. The endogenous and microbial enzyme systems were deeply studied in the 1990s, with special focus on its role in the processing and quality of these meat products (Toldrá 2002). The main enzymatic processes that take place during the processing of meat products, and are directly involved on flavor development, are briefly schematized in Figure 61.2 and described below.

61.2.1 Proteolysis

Proteolysis consists in the enzymatic hydrolysis of myofibrillar and sarcoplasmic proteins. This hydrolysis is brought about by the combined action of muscle proteinases (cathepsins and calpains) and exopeptidases. In fermented meat products, like dry fermented sausages, starter proteases also play an important role on proteolysis. Proteolysis contributes to the consistency of the product by the degradation of the myofibrillar structure and to its taste through the accumulation of small peptides and free amino acids. These amino acids directly contribute to flavor or indirectly as precursors of flavor compounds through amino acid degradation reactions (Toldrá and Flores 1998). The extent of proteolysis is variable and mainly depends on the raw materials and processing conditions and, in the case of fermented meats, the type of starters added (Toldrá 2002).

61.2.2 Lypolysis

Lipolysis consists in the enzymatic hydrolysis of triacylglycerols and phospholipids. Muscle and adipose tissue lipases as well as muscle phospholipases, respectively, are responsible for these hydrolysis (Toldrá and Navarro 2002). In fermented meat products, like dry fermented sausages, lipases from microbial starters also play an important role on lypolysis. The generation of free fatty acids as final products is very important for flavor because they will act as substrates for further oxidative reactions for the formation of volatile compounds with aroma properties (Flores and others 1998).

61.2.3 Nucleotides Breakdown

The original ATP present in living muscle is degraded very rapidly, reaching negligible values in just a few hours. This degradation is complex and involves numerous enzymes. Main changes are observed during the first days postmortem. In this way, intermediate degradation compounds, like ADP and AMP, disappear within 24 h postmortem. Other nucleotides, like IMP (inosin monophosphate), shows maximum levels at 1 day postmortem and then slightly decrease. Inosine and hypoxanthine, which are final products of the nucleotides breakdown, increase up to 7 days postmortem (Batlle and others 2001).

61.2.4 Glycolysis

Living muscle contains certain amounts of glycogen and glucose. These carbohydrates are rapidly hydrolyzed in postmortem muscle producing lactic acid as end product. The accumulation of lactic acid in the muscle produces a pH drop towards acid values around 5.3–5.8. The contribution of glycolysis is restricted to few hours in postmortem meat but is very important in fermented meats where sugar is added for microorganisms to grow. In this case, glycolysis is mainly due to the enzymatic system of lactic acid bacteria that produces high amounts of lactic acid as end product (Demeyer and Toldrá 2004).

61.3 TASTE CONTRIBUTION OF NONVOLATILE COMPOUNDS

Nonvolatile compounds are formed during meat processing. Mechanisms like glycolysis, proteolysis, and lipolysis produce a good number of nonvolatile compounds with important flavor properties that play an important role in the taste impression of the product (Toldrá and Flores 2004). Endogenous muscle enzymes are responsible for most of these reactions and their extent will depend on the type of process as reflected in Table 61.1. In the case of cooked products, the processing time is short but the increase of temperature during cooking accelerates the enzymatic action although for a few hours. On the other hand, a good number of nonvolatile compounds are generated in dry-cured ham because of its long ripening time, sometimes longer than 1 or even 2 years.

TABLE 61.1 Relative Importance of Taste Contributor Substances Present and/or Generated During the Processing of Different Meat Products.[a]

Substances	Cooked Ham	Dry-Cured Ham	Dry-Fermented Sausage
Free amino acids	+	+++	++
Peptides	+	+++	++
Nucleotides	++	+	+
Lactic acid	+	−	+++
Long-chain free fatty acids	+	+++	++
Short-chain free fatty acids	−	+	++
Carbohydrates	++	−	+
Inorganic salts	+	+++	++
Spices/condiments	+	−	+++

[a]Relative intensity: no intensity (−), poor (+), medium (++), high (+++).

TABLE 61.2 Taste Contribution of Compounds Present and/or Generated During the Processing of Cured Meat Products.

Sour	Sweet	Bitter	Salty	Umami
Lactic acid	Carbohydrates	Phenylalanine, tryptophane, tyrosine	Inorganic salts (NaCl)	Nucleotides, nucleosides
Dipeptides containing glutamic or aspartic acids	Glycine, alanine	Peptides with hydrophobic amino acid side chain		Glutamate, aspartate
Short and long-chain free fatty acids				

In the case of fermented meat products, there is a combined action between muscle and microbial enzymes and the accumulation of end products depending on the type of product, especially the pH drop rate and length of the process.

The main products resulting from glycolysis are organic acids, being the major products lactate and acetate, that contribute to the sour taste of the product (see Table 61.2). This pH drop is usual in fermented meats when starters are used, mainly lactic acid bacteria. It positively affects color development, texture, and homogeneity of drying. However, an excessive production of these acids may impart unpleasant flavors due to the partial masking of global aroma by the sourness. Some sweetness may be appreciated if some nonhydrolyzed carbohydrates are remaining. Lipolysis, either from endogenous meat enzymes or from starter cultures, generates free fatty acids and di- and monoacylglycerols. The contribution of free fatty acids to the sour taste is rather poor because of the low concentration of these compounds for effective perception. It would be only significant in dry-cured ham, where the generation of long chain fatty acids is quite important (see Table 61.2). The further oxidation of the free fatty acids generates many different compounds responsible of the aroma of the product as will be later described. While lipolysis is mainly due to endogenous enzymes present in the muscle and adipose tissue, the lipid oxidation process is due to either microbial or chemical action and thus they should be considered as different processes.

Many yeasts and molds, usually found in fermented sausages, may contribute to the fermented flavor. They contain lypolytic enzymes that contribute to flavor through the generation of carbonyl compounds. Molds and yeast are also able to oxidize lactic acid in the presence of oxygen. In addition, a mold layer on the surface of the sausage contributes to air drying by reducing available oxygen in the sausage.

Polypeptides, peptides, and free amino acids are the main products from proteolysis. They have shown an improvement in meat taste during storage (Nishimura and others 1988; Spanier and Miller 1993) and are also very important for taste development in meat products (Nishimura and others 1988; Kato and others 1989). The extend and profile of proteolysis depends on the type of product and its pH. So, cathepsin D is very important in acid products, like dry-fermented sausages, and some important proteins as myosin and actin are broken down to fragments with 135 and 38 kDa, respectively (Demeyer and Toldrá 2004). In products with mild pH values, like dry-cured ham, initial proteolysis is exerted by calpains and cathepsins B and L. Major myofibrillar proteins are also broken down to oligopeptides, small tri- and dipeptides and free amino acids (Toldrá and others 2000). A tripeptide containing Glu, Val, and Asp and some dipeptides like Ile-Val, Leu-Glu, Ile-Asp, Ala-Met, Gly-Glu, Glu-Arg, Pro-Leu, Gly-Ser, Asp-Val,

and Ser-Lys have been isolated and identified at the end of the process (Sentandreu and others 2003). When hydrophobic peptides and/or hydrophobic amino acids are predominant, a bitter taste and off-flavors are typically perceived (Aristoy and Toldrá 1995). In fact, free amino acids are very important for taste (see Table 61.2). Hydrophobic amino acids have a bitter taste while other amino acids like alanine and glycine may elicit sweet taste (Nishimura and Kato 1988). Sodium glutamate and aspartate contribute to the umami taste but also to sour taste when they are in dissociated state (Kato and others 1989). An example of free amino acids generation for different meat products is shown in Table 61.3 where noticeable differences may be observed due to the different processes. Large amounts are produced in dry-cured ham due to the longer processing time while few amounts are produced in cooked ham where processing is very short. Dry fermented sausages show intermediate amounts. Some unpleasant tastes such as bitter-like or metal aftertaste have been reported as a consequence of an excessive protein hydrolysis, when the proteolysis index is higher than 29–30% (Careri and others 1993; Virgili and others 1995).

The activity of hydrolytic enzymes may be altered by heating in cooked meat products. In fact, heating causes structural and functional changes in meat proteins that generate additional peptides and amino acids, that may have their own flavor, and also react with reducing carbohydrates to originate new taste and aroma compounds via Maillard reaction and Strecker degradation (Spanier and others 2004).

Amino acids and their degradation products may contribute to the taste of the meat product. In the case of fermented meats, free amino acids could be further decarboxylated, deaminated, or even further metabolized depending on the type of microbial flora developed in the product (Toldrá and others 2001). So, some rise of pH may be expected if ammonia is formed and thus, sausage taste would be milder in sourness. In addition,

TABLE 61.3 Generation of Free Amino Acids (Expressed in mg/100 g) in Cooked Ham, Dry-Cured Ham and Dry-Fermented Sausage.

Amino Acids	Cooked Ham	Dry-Cured Ham	Dry-Fermented Sausage
Aspartic acid	14.6	300.4	43.4
Glutamic acid	36.9	493.2	180.6
Serine	8.0	247.9	49.4
Asparagine	1.2	26.1	22.5
Glycine	6.5	208.8	71.9
Glutamine	0	0	32.5
Tyrosine	5.4	169.7	4.4
Proline	4.1	285.4	47.8
Alanine	12.8	374.8	137.7
Arginine	10.4	226.9	64.4
Threonine	5.8	276.6	50.1
Valine	4.2	312.1	94.0
Methionine	3.0	132.3	54.2
Isoleucine	4.1	216.5	75.1
Leucine	4.0	339.8	138.9
Phenylalanine	4.9	207.1	83.3
Tryptophan	0	30.7	13.3
Histidine	7.8	94.8	28.9
Lysine	9.5	731.4	58.1

Source: Data from Toldrá and others (1995), Flores and others (1998).

aminopeptidases would be less inhibited by acid pH values and would generate higher levels of free amino acids. Therefore, an equilibrium between acid production and taste is required in fermented meat products, taking into account that ammonia production by certain yeasts like *Debaryomyces hansenii* may neutralize final acidity and enhance sausage taste (Durá and others 2004). Some umami taste may be due to nondegraded nucleotides, like 5′-inosin monophosphate and 5′-guanosin monophosphate which is specially important in cooked meats (see Table 61.2). Finally, just mention that salty taste is due to the added inorganic salts, usually sodium chloride, that is a typical curing agent in meat products. The contribution of spices and condiments is very important in dry fermented sausages where they are typically added.

61.4 MECHANISM OF GENERATION OF VOLATILE COMPOUNDS AND CONTRIBUTION TO THE AROMA OF MEAT PRODUCTS

The compounds that act as precursors of meat flavor have been classified into two categories; water soluble compounds and lipids. However, as indicated above, the generation of these compounds depends on the processing conditions. In this sense, the effect of heating, smoking, and spices added to meat products, are responsible of many flavor differences among cured meat products through reactions that lead to meat aroma volatiles. The main reactions that occur during the processing of meat products are: carbohydrate degradation, amino acid degradation reactions (decarboxylation, deamination, transamination), Maillard reactions, Strecker degradation, degradation of ribonucleotides, thiamin degradation, and lipid degradation. The contribution of these reactions to aroma formation depends on the specific processing technology for each meat product (see Table 61.4). In general, these reactions are quite more complex due to secondary reactions that can occur between the products resulting from the initial reactions giving a high number of aromatic compounds.

61.4.1 Carbohydrate Degradation

The degradation of carbohydrates takes place by heating or fermentation. The heating process produces caramelized flavors due to the formation of high odor compounds such as furan derivatives, carbonyls, alcohols, and aliphatic and aromatic hydrocarbons

TABLE 61.4 Relative Importance of Mechanism of Aroma Formation During the Processing of Different Meat Products.[a]

Mechanisms	Frankfurter	Cooked Ham	Dry-Cured Ham	Dry-Fermented Sausage
Carbohydrate degradation (heating or fermentation)	+	+	−	+++
Microbial amino acid degradation reactions	−	−	−	++
Maillard reactions	+++	+++	+	+
Strecker degradation	+++	+++	++	+
Degradation of ribonucleotides	+++	+++	++	++
Thiamin degradation	++	++	++	++
Lipid degradation	+++	+++	+++	+++

[a]Relative intensity: no effect (−), poor (+), medium (++), high (+++).

(Mottram 1991). These sugar caramelization reactions are mainly produced in small surface areas of roast and grilled meat that have been dehydrated. Therefore, the contribution of the sugar degradation reaction in meat products such as frankfurters and cooked ham will depend on the further heating treatment applied. However, the concentration of sugars in meat products is low except in fermented products were they constitute the substrate for the fermentation process being the second route for carbohydrate degradation. In this sense, the fermentation process release compounds of low molecular weight such as diacetyl, acetoin, butanediol, acetaldehyde, ethanol, and acetic acid. The generation of the specific volatile compounds during the carbohydrate fermentation depends on the starter used in the processing of dry fermented sausages. The generation of compounds such as diacetyl, acetoin, or butanediol impart a butter and yogurt aroma in fermented sausages. Sugar degradation is an important source of di- and tricarbonyls which may react subsequently with amino acids in Strecker reactions (MacLeod and Seyyedain-Ardebili 1981).

61.4.2 Amino Acid Degradation Reactions

The degradation of amino acids can be produced by the enzymatic activity present in the meat products. This is the case of fermented meat products where the microbial population is responsible of reactions such as the degradation of amino acid side chain, decarboxylation, oxidative deamination, and transamination (Toldrá and others 2001). The side chain degradation for tyrosine and tryptophane leads to phenol and indole formation (Molimard and Spinnler 1996) producing compounds such as 3-methylindole (skatole) responsible of unpleasant odors in meat products. On the other hand, the microbial decarboxylation of amino acids produce biogenic amines (Ordoñez and others 1999) that can affect negatively the flavor and safety of the meat product. The oxidative and nonoxidative deamination of amino acids produce keto acids that can be also transformed to aldehyde by descarboxylation and then, this aldehyde can be either reduced to the corresponding primary alcohol or oxidized to an acid. The generation of aromatic compounds from methyl branched amino acids in dry fermented sausages is mainly due to the action of staphylococci and lactic acid bacteria (Berdagué and others 1993; Stahnke 1995, 1999; Schmidt and Berger 1998). The catabolism of branched amino acids such as valine, leucine, and isoleucine generates 2- and 3-methylbutanal, 2- and 3-methylbutanol, 2- and 3-methylpentanoic acids, respectively. Dimethyldisulfide is catabolyzed from cysteine and benzeneacetaldehyde from phenylalanine. These sulfur compounds are important contributors to meat flavor because of their low threshold values.

61.4.3 Maillard Reactions

These reactions between reducing sugars and amino compounds are responsible of many flavor compounds in cooked foods (Mottram 1991). The reaction rate increases with temperature and is associated with low moisture levels. The flavor compounds are mainly generated at the temperature usual of cooking processes. The first step of the reaction is the addition of the carbonyl group of the open chain form of a reducing sugar and the amino group of an amino acid, peptide, or other compound with a primary amino group, to give a glycosylamine. The further elimination of water gives a Schiff's base that with subsequent rearrangement forms Amadori and Heyns intermediates that by themselves do not contribute to flavor although they are precursors of flavor

compounds (furanones derivatives, furfurals, dicarbonyl compounds, and others). All these Maillard products can experience further reactions with other reactive compounds; amines, amino acids, hydrogen sulfide, thiols, ammonia, acetaldehyde, and other aldehydes, leading to many important flavor compounds including heterocycles (pyrazines, oxazoles, thiophenes, thiazoles, and others). Furfurals and furanones, generally impart caramel-like, sweet, fruity characteristics to foods. Probably, they are minor contributors to meat-like flavors however they are important intermediates to other flavor compounds.

61.4.4 Strecker Degradation

The Strecker degradation of amino acids consists in the oxidative deamination and decarboxylation of an α-amino acid in the presence of a dicarbonyl compound. The dicarbonyl compounds arise from sugar degradations or Maillard reactions. The reaction produces an aldehyde containing one fewer carbon atom than the original amino acid and an α-aminoketone. Thus, acetaldehyde is formed from alanine, propanal from α-aminobutyric acid, 3-methylbutanal from leucine, 2-methylbutanal from isoleucine, 2-methylpropanal from valine, phenylacetaldehyde from phenylalanine, and methional from methionine (MacLeod and Seyyedain-Ardebili 1981). Furthermore, the Strecker degradation of sulfur containing amino acids such as methionine, cysteine, and cystine leads to the production of hydrogen sulfide, ammonia, and acetaldehyde. These compounds are important as reactive intermediates for the formation of sulfur compounds characterized by low threshold values and therefore, a high aromatic impact in meat products (Shahidi and others 1986).

61.4.5 Degradation of Ribonucleotides

The contribution of this reaction to the generation of flavor compounds in meat is by the production of ribose from inosine monophosphate and other ribonuclotides. This ribose takes part in the Maillard reaction generating important flavor compounds. The reaction of 4-hydroxy-5-methyl-3(2H)furanone that comes from the degradation of ribose, with hydrogen sulfide produces a complex mixture of compounds possessing roasted meat odors. These compounds are identified as mercapto-substituted furan and mercapto-substituted thiophen derivatives (MacLeod and Seyyedain-Ardebili 1981).

61.4.6 Degradation of Thiamin

The degradation of thiamin during the cooking process produces a number of aromatic compounds including furans, furanthiols, thiophenes, thiazoles, and aliphatic sulfur compounds (Mottram 1991). These compounds have been reported to possess roasted meat odors and have been found in cooked meats (MacLeod and Seyyedain-Ardebili 1981).

61.4.7 Lipid Degradation

The oxidation of unsaturated fatty acids is responsible for the generation of flavor compounds. The oxidation process can be due to the effect of temperature during meat cooking, autoxidation, and autoxidation during dry-curing. The thermally induced oxidation is an important route to aroma volatiles during cooking (Mottram 1998), the autoxidation during storage of fatty foods produce undesirable flavors associated with

rancidity (Gray and others 1996; StAngelo 1996) and the drying process during dry-curing produce many volatile compounds responsible for the cured flavor (Toldrá and Flores 1998; Gandemer 1999, 2002). The mechanism of autoxidation is free radical in nature and the reaction has been widely studied (Frankel 1980). Phospholipids contain higher amounts of polyunsaturated fatty acids than triacylglycerols, making them more susceptible to oxidation. The contribution of phospholipids to cooked meat aroma has been demonstrated (Mottram and Salter 1989; Mottram and Whitfield 1995) also their contribution to the development of the characteristic aroma in the processing of dry-cured ham (Flores and others 1985, 1987). Lipid oxidation accounts for the generation of nonbranched aliphatic compounds such as alkanes, alkenes, ketones, aldehydes, alcohols, carboxylic acids, esters, and several furanic cycles. The contribution of alkanes to flavor is almost irrelevant due to their high thresholds. The flavor of alcohols was considered unimportant in comparison to other carbonyl compounds. The straight chain primary alcohols are relatively flavorless but as the carbon chain increases, the flavor becomes stronger (Shahidi and others 1986) giving greenish, woody, and fatty floral notes. C3 and C4 aldehydes exert sharp and irritating flavor, intermediate (C5–C9) have green, oily, and fatty flavors and the higher (C10–C12) exert citrus flavor (Forss 1972).

61.5 CONTRIBUTION OF SPICES TO THE AROMA OF MEAT PRODUCTS

Although not a mechanism of formation of flavor compounds, spices constitute a source of many volatile compounds that will impart a specific characteristic to the meat product. In this sense, the spices and condiments added in the manufacture of meat products contribute to a particular flavor, depending on local traditions. In fact, there are many specific flavors due to the high number of available aromatic plants such as pepper, paprika, mustard, nutmeg, cloves, oregano, rosemary, thyme, garlic, onion, and so on. These compounds have a high impact on the aroma of fermented products (Ordoñez and others 1999). For instance, the high content of terpene hydrocarbons or sulfur compounds found in the headspace of dry fermented sausages, comes from pepper or garlic, respectively. Dry-cured ham is not normally condimented with spices and only salt and nitrate are added in its formulation.

61.6 CONTRIBUTION OF SMOKING TO THE FLAVOR OF SMOKED MEAT PRODUCTS

The practice of smoking of meat and meat products produces a drying effect, imparts desirable flavor and color of the meat and protects the meat product from rancidity and spoilage (Ellis 2001). Nowadays, meat smoking consist on the addition of vaporous or liquid smoke to meat or meat products (Fessmann 1995). The major contribution of liquid smoke to the headspace of meat products are phenols, cyclopentenones and furans (Knowles and others 1975; Guillen and others 1995). Phenol and methoxyphenol derivatives are originated mainly from the pyrolysis of lignin which accounts for 25% of the composition of wood. Cyclopentanones are produced from dicarboxylic acids by decarboxylation and ring formation, while furans probably come from the degradation of glucose, a thermal degradation product of cellulose (Toth and Potthast 1984). The characteristic smoke flavor has been attributed to phenols (Fiddler and others 1970).

61.7 IDENTIFICATION AND RELEVANCE OF VOLATILE COMPOUNDS TO THE FLAVOR OF MEAT PRODUCTS

Many volatile compounds have been identified in cooked ham (Baloga and others 1990; De Winne and Dirinck 1997; Guillard and others 1997), frankfurters (Chevance and Farmer 1999a,b; Chevance and others 2000; Estevez and others 2005), dry-cured ham (Berdague and others 1991; Buscalhion and others 1994; Bolzoni and others 1996; Flores and others 1997; Ruiz and others 1998, 1999; Andres and others 2002; Timon and others 2002; Carrapiso and others 2003; Garcia-Esteban and others 2004; Sanchez-Peña and others 2005) and fermented products (Berdagué and others 1993; Stahnke 1994, 1995; Viallon and others 1996; Ansorena and others 2001; Stahnke and others 2002; Muguerza and others 2003; Benito and others 2004; Olesen and others 2004; Tjener and others 2004a,b; Flores and others 2004, 2005; Herranz and others 2005; Hierro and others 2005; Bolumar and others 2006). The volatile compounds identified in these meat products belong to the following classes: alkanes, alkenes, aldehydes, ketones, alcohols, aromatic hydrocarbons, carboxylic acids, esters, terpenes, sulfur compounds, furans, pyrazines, amines, and chloride compounds. Although many compounds are found in the four meat products, its flavor is completely different depending on its processing. Different pathways in the processing technology are responsible for the formation of these volatile compounds. However, the impact of an odor component on the total aroma depends on a number of factors, such as odor threshold, concentration, solubility in water or fat, and temperature as reported for dry-cured ham flavor (Flores and others 1998).

Solid-phase micro-extraction (SPME) is a recent extraction technique very useful for the study of aroma compounds in foods (Steffen and Pawliszyn 1996) and specially, in meat products (Ruiz and others 1998; Gianelli and others 2002; García-Esteban and others 2004; Marco and others 2004). Typical SPME gas chromatograms of cooked ham and frankfurter is shown in Figures 61.3*a* and 61.3*b*, respectively. It is remarkable the high number of volatile compounds detected, for example compounds 1 to 9 were detected in both cured meat products (see Table 61.5). However, compounds C to F were only detected in frankfurter, typical compounds from the smoking treatment. On the other hand, typical SPME gas chromatograms of dry-fermented sausages and dry-cured ham are shown in Figures 61.4*a* and 61.4*b*, respectively. Several of the most abundant compounds are indicated in Table 61.6 being two compounds, three (acetic acid) and six (hexanal) detected in high proportion in both meat products however, the chromatograms were completely different.

In many cases, the study of volatile compounds in meat products has been focused on the most abundant compounds without taking into consideration their contribution to the overall aroma. The study of odor impact compounds in meat products has been done through olfactometry techniques (Toldrá and Flores 2004).

The odor-active compounds detected in dry meat products including their odor description and possible origin are shown in Table 61.7. In dry-cured ham, several sulfur compounds have been detected and contribute to dry-cured aroma (Carrapiso and others 2002a,b; Carrapiso and García 2004). It is remarkable that these sulfur compounds are in very low proportions in dry-cured ham but its effect is due to their low thresholds (Shahidi and others 1986). On the other hand, aldehydes that result from amino acid degradation and lipid oxidation are contributing to the aroma of dry-cured ham as several ketones that also come from lipid oxidation. Only one ester compound has been described

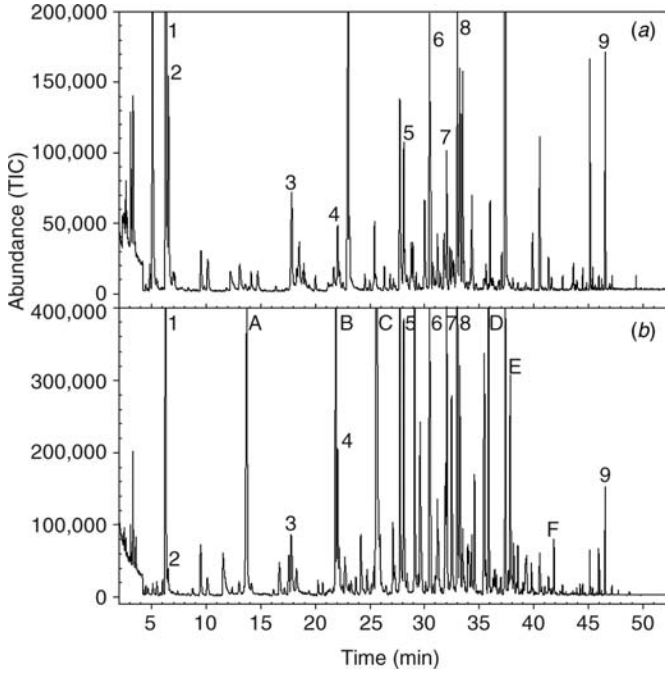

Figure 61.3 *Typical gas chromatogram of the volatile compounds of cooked ham (a) and frankfurter (b). Chromatograms obtained by GC-MS using the SPME fiber (75 μm Carboxen/PDMS) after sampling the headspace for 2 h at 30° C. The numbers and letters represent compounds identified and listed in Table 5.*

as contributor to the aroma of dry-cured ham. However, the presence of ester compounds in dry-cured ham depends on the processing conditions (Toldrá and Flores 1998) because they have been isolated in higher proportions in Parma (Italian) than in Iberian or Serrano (Spanish) dry-cured hams.

TABLE 61.5 Major Compounds Detected in the Headspace of Cooked Cured Meat Products by Solid Phase Microextraction (SPME).

Figure Identification[a]	Cooked Ham	Frankfurter
1	2-Butanone	2-Butanone
2	Ethyl acetate	Ethyl acetate
3	3-Hidroxy-2-butanone	3-Hidroxy-2-butanone
4	Hexanal	Hexanal
5	α-Pinene	α-Pinene
6	β-Phellandrene	β-Phellandrene
7	3-Carene	3-Carene
8	Limonene	Limonene
9	Caryophyllene	Caryophyllene
A		1-Hidroxy-2-propanone
B		1-Hidroxy-2-butanona
C		Furfural
D		Phenol
E		2-Methoxy-phenol
F		2-Methoxy-4-methyl-phenol

[a]Numbers and letters correspond to those indicated in Figure 61.3.

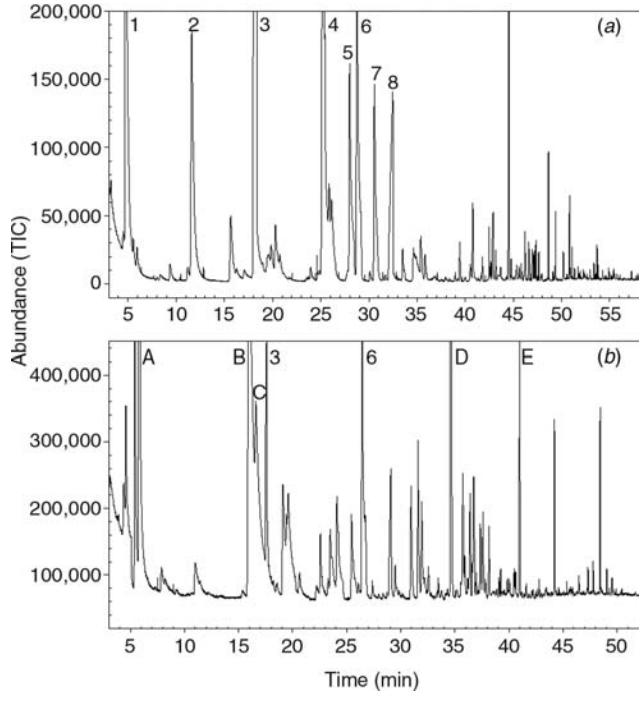

Figure 61.4 *Typical gas chromatogram of the volatile compounds of dry-fermented sausage (a) and dry-cured ham (b). Chromatograms obtained by GC-MS using the SPME fiber (75 μm Carboxen/PDMS) after sampling the headspace for 3 h at 30° C. The numbers and letters represent compounds identified and listed in Table 6.*

On the other hand, many ester compounds have been detected as odor active compounds in dry fermented sausages (Stahnke 1994; Meynier and others 1999) followed by several aldehydes, one ketone, one carboxylic acid, one sulfur compound, one pyrrol, and one alcohol (Table 61.7). The contribution of esters compounds to the aroma of fermented sausages is very important even though their concentrations are

TABLE 61.6 Major Compounds Detected in the Headspace of Dry Cured Meat Products by Solid Phase Microextraction (SPME).

Figure Identification[a]	Dry-Fermented Sausages	Dry-Cured Ham
1	Ethanol	
2	Ethyl acetate	
3	Acetic acid	Acetic acid
4	3-Methyl butanol + 2-methyl butanol	
5	Ethyl butanoate	
6	Hexanal	Hexanal
7	Ethyl 2-hidroxy propanoate	
8	Butanoic acid	
A		Isopropanol
B		3-Methyl-butanal
C		2-Methyl butanal
D		Branched chain hydrocarbon
E		Nonanal

[a]Numbers and letters correspond to those indicated in Figure 61.4.

TABLE 61.7 Odor-Active Compounds with Odor Description and Possible Origin of Compounds in Dry-Cured Meat Products.

	Dry-Cured Ham[a]			Dry-Fermented Sausage[b]		
Group of Compounds	Compound	Odor[c]	Origin[d]	Compound	Odor	Origin
Sulfur compounds	2-methyl-3-furanthiol	Cured-ham-like, toasted	Thia	Methional	Cooked potato	Aac
	methanethiol	Rotten eggs, meat	Aac			
	hydrogen sulfide	Boiled eggs, sewage	Aac			
	Methional	Potato-like	Aac			
Aldehydes	2-methyl-propanal	Fruity, pungent	Aac	3-methyl-butanal	Sour cheese	Aac
	3-methyl-butanal	Fruity, almond-like	Aac	2-methyl-butanal	Nailpolish	Aac
	Hexanal	Green	Lipid ox	Hexanal	Green leaves	Lipid ox
	(E)-2-Hexenal	Fruity, green	Lipid ox	Heptanal	Potatoes	Lipid ox
	(Z)-3-Hexenal	Fruity	Lipid ox	decanal	Cucumber, dry grass	Lipid ox
	(E)-2-Nonenal	Fatty, leather-like	Lipid ox			
Ketones	2-heptanone	Nutty	Lipid ox	2,3-butandione	Butter	Ferm
	1-penten-3-one	Rotten, fruity	Lipid ox			
Esters	ethyl-2-methylbutanoate	Fruity, apple-like	Esterific	Propyl acetate	Souris apple, candy	Esterific
				Ethyl isobutanoate	Pineapple, fruit	
				Ethyl butanoate	Fruit candy	
				Ethyl 2-methylbutanoate	Sweet pineapple	
				Ethyl 3-methylbutanoate	Chutney, spicy	
Pyrrol	2-acetyl-1-pyrroline	Roasty, popcorn	Aac	2-acetyl-1-pyrroline	Roasty, popcorn	Aac
Acids				3-methyl-butanoic acid	Sweaty socks	Aac
Alcohols	1-Octen-3-ol	Mushroom	Lipid ox	1-octen-3-ol	Mushroom	Lipid ox
Other	Sotolone	Seasoning-like	Spices	Myrcene	Lemon, fruity	Spices
				Limonene	Menthol	Spices
				Terpinolene	Fruity, eucalyptus	Spices

[a]Carrapiso and others (2002a,b), Carrapiso and Garcia (2004).
[b]Meynier and others (1999), Stahnke (1994), Blank and others (2001).
[c]Odor: odor detected by assessors in the cited references.
[d]Possible origin of compounds derived from: Thia: degradation of thiamin; Aac: degradation of amino acids; Lipid ox: oxidation of lipids; Esterific: esterification by microbial or chemical action; Ferm: fermentation; Spices; added spices.

TABLE 61.8 Odor-Active Compounds with Odor Description and Possible Origin of Compounds in Cooked Cured Meat Products.

	Frankfurter[a]			Cooked Ham[b]		
Group of Compounds	Compound	Odor[c]	Origin[d]	Compound	Odor	Origin
Sulfur compounds	Methional	Potatoes, roasted meat	Aac	Methional	Cooked potatoes, grass	Aac
	2-Methyl-3-methylthiofuran	Roasted meat, grilled fat	Thia, 5′Ribo	Dimethyl disulfide	Fermented, plastic	Aac
	Dimethyl trisulfide	Old cooking smell, gassy	Aac	Allyl isothiocyanate	Sulphurous, cheese	Spices
	2-Furanmethanethiol	Roasted, coffee beans,	Thia, 5′Ribo			
	2-Methyl-3-methyldithiofuran	Meaty, roasty	Thia, 5′Ribo			
	2-Acetyl-thiazoline	Roasted meat, popcorn	Thia			
Ketones	2,3-Butandione	Buttery	Meat/smoke			
	1-Octen-3-one	Mushrooms	Thermal Ox			
Acids				3-Methyl-butanoic acid	Rotten peas, cheese	Aac
Furans	2-Acetylfuran	Raw potatoes, stale	Thermal Ox, Maillard			
Alcohols	2-Methoxyphenol	Smoky, frankfurter,	Smoke			
	2-Methoxy-4-methylphenol	Smoky	Smoke			
	2-Methoxy-4-propylphenol	Stale, gassy	Smoke			
	2,6-Dimethoxyphenol	Smoky, frankfurter	Smoke			
Other	1,8-Cineole	Medicinal, cough syrup	Spices	1,8-Cineole	Eucalyptus, mint	Spices
	Linalool	Green, pine needles	Spices	Linalool	Orange, flower	Spices
	α-Pinene	Flowers, carnation	Spices	L-Carvone	Mint, chewing gum	Spices
				Cinnamaldehyde	Cinnamon	Spices
				Menthol	Mint	Spices

[a]Chevance and Farmer (1999a,b).
[b]Guillard and others (1997).
[c]Odor: odor detected by assessors in the cited references.
[d]Possible origin of compounds derived from: Thia: degradation of thiamin; Aac: degradation of amino acids; Thermal ox: thermal oxidation of lipids; 5′Ribo: breakdown of 5′-ribonucleotides; Maillard: degradation of carbohydrates in the Maillard reaction; Spices; added spices; Smoke; incorporated smoke.

very low; they are easily detected by sniffing adding fruity notes and masking rancid odors (Stahnke 1994). It is important to remark the diverse pathways originating the same volatile compound. Sulfur compounds such as methional, dimethyl disulfide, and methylpropyl disulfide have been found in the headspace of fermented sausages and they can arise from the Strecker degradation of cysteine and methionine, or from the garlic added as a spice (Viallon and others 1996). On the other hand, compounds such as acetic acid and ethanol detected in high proportions (peaks 1 and 3 in Fig. 61.3*a*), can also be produced in the catabolism of lipids or amino acids and some methyl compounds also generated in the degradation of branched amino acids by Strecker degradations. Blank and others (2001) indicated that pyrrol compounds seems to play an important role in fermented meat products by contributing to a strong roasty note. However, its formation is not clear as they can be producuced by the Strecker degradation of proline or also by sugar degradation and microbial origin. The content in volatile compounds depends on the dry fermented sausage process. In the case of Italian and Spanish sausages, they mainly contain the following dominant compounds: terpenes (from spices), ketones and aldehydes (from lipolysis and lipid oxidation), and esters. However, certain low acid sausages contain aldehydes, ketones, alcohols and esters, and low quantities of N-containing volatiles, indicating a low proteolysis in the product. On the other hand, sausages of medium acidity (pH = 5.1–5.3) contain aldehydes and ketones (constituents of the 60% of total volatile), furans, sulfur compounds, pyrazines, and amines, indicating a high proteolysis in the product (Ordoñez and others 1999).

The differences in volatile composition of uncured and cured cooked pork has been studied by several reports (Ramarathnam and others 1991, 1993) in order to establish the effect of curing in the development of cured cooked flavor. Different reports have studied the volatile composition of cooked ham (Baloga and others 1990) and the effect of refrigerated storage on the volatile compounds (De Winne and Dirinck 1997). However, few reports have studied the odor active compounds in cooked cured meat products (Table 61.8) (Guillard and others 1997; Chevance and Farmer 1999a,b). Many volatile compounds identified in these products are originated from smoke and seasonings while others come from reactions occurring in the meat as described above. Therefore, it is necessary to know the processing conditions and additives added to the meat product. In the study of odor active compounds in cooked cured meat products, several terpenes were identified from cooked ham and frankfurters (Table 61.8). Moreover, several compounds derived from liquid smoke played a major contribution to the aroma of frankfurter (Chevance and Farmer 1999) being phenols the most important group of compounds. On the other hand, the compounds responsible of many meaty, roasted and grilled notes in frankfurters were sulfur-containing compounds. However, different structures of sulfur compounds have been detected in frankfurters that can be due to different cooking procedures applied (Chevance and Farmer 1999a). This is the case of oven cooking that can produce higher temperatures and lower moisture levels on the surface favoring the Maillard reaction in comparison to cooking in a water bath. On the other hand, few compounds have been described as odor active compounds in cooked ham, apart from terpenes from the spices added, the sulfur-compounds are contributing to the aroma of cooked ham and a branched acid originated from the Strecker degradation of amino acids (Guillard and others 1997) (Table 61.8). It is important to remark that the refrigerated storage of cooked cured products can modify the volatile composition due to the presence of lipid oxidation and fermentation phenomenon that will generate typical fermentation products such as methyl branched alcohols and aldehydes, and oxidation products such as unsaturated aldehydes (De Winne and Dirinck 1997) that are not present in the fresh cooked ham.

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62

Sensory Quality of Meat Products

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62.1 Introduction 303
62.2 Effects of Processing Technology on Sensory Attributes 304
62.3 Processed Meat Products 305
62.3.1 Roast Beef 305
62.3.2 Bacon 306
62.3.3 Frankfurters 308
62.3.4 Cooked Ham 312
62.3.5 Dry-Fermented Sausage 315
62.3.6 Dry-Cured Ham 316
References 323

62.1 INTRODUCTION

There is a trend in the last decades towards an increased demand of meat products due to many factors including changes in consumer lifestyles, such as the demand for easy-to-prepare products with convenient and smaller portions. There is a wide variety of processed meat products and, in fact, its quality varies according to the ingredients and additives used in the formulation as well as the type of processing (Flores and Toldrá 1993). So, the sensory characteristics vary according to the type of product but, even for a given product, particular

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characteristics may change depending on the geographical area (i.e., addition of certain spices), physical appearance (form, diameter of cross-section, size of fat particles, specific color), extent of drying and/or ripening, application of smoking, presence of external molds, and so on (Toldrá 2002, 2004a). The contribution of volatile compounds to the aroma of meat products has been deeply studied. In fact, the contribution of volatile compounds to flavor depends on many factors such as particular odor characteristics (i.e., odor threshold), concentration in the product, solubility in water or fat and temperature (Toldrá and Flores 2004). Something similar happens with the relation between nonvolatile components such as peptides amino acids and nucleotides and product flavor (Spanier and Miller 1993). Many studies have related the volatile and nonvolatile compounds with the sensory charactersitics of the meat products by using multivariate statistical methods.

The sensory attributes of meat products may differ depending on the type of product, specially due to important processing differences. In this chapter, the main sensory characteristics of some selected beef and pork meat products will be presented and its evaluation discussed.

62.2 EFFECTS OF PROCESSING TECHNOLOGY ON SENSORY ATTRIBUTES

There are many types of meat products, most of them treated with salt and sodium nitrite and subjected to either cooking, smoking, or drying. In general, there are several important groups: wet-curing, pickle-curing, and dry-curing (Flores and Toldrá 1993). In wet-curing the additives are mixed with the minced meat and fat, and ice to cool the meat paste and then stuffed into casings. In pickle-curing the additives are dissolved in water forming a brine that penetrates into the meat by either soaking or injection. In both cases, the meat products are cooked and, optionally, smoked. In dry-curing, the curing additives can be either rubbed onto the outer surface of the ham and left for ripening and drying, as is the case for dry-cured ham (Toldrá 2004b), or mixed with the minced meat and fat, stuffed into casings and left to ferment and dry, that is the case for dry-fermented sausages. These products can also be optionally smoked (Demeyer and Toldrá 2004).

The acceptability of cured meat is largely dependent upon its flavor: however, very little is known about the chemical mechanisms involved in the reactions leading to the formation of cured meat flavor and furthermore, there are certain difficulties in the elucidation of the compounds responsible for this flavor (Ramarathnam 1998). In fact, many volatile compounds have been isolated and identified from cured pork meats although no compound has been identified yet as responsible of cured meat flavor (Shahidi and others 1986; Flores and others 1998; Ramarathnam 1998).

Curing additives have several roles, some of them closely related with sensory characteristics, as briefly described here. The main functions of salt are its contribution to taste and preservation as well as the enhancement of the functional properties of meat proteins. Sugar produces an enhancement of the flavor of the cured product and constitutes a substrate for fermentation while the reducing agents (ascorbates and erythorbates) accelerate the development of the characteristic pink color of cured meat. Nitrite exerts an antimicrobial effect by preventing the growth of *Clostrodium botulinum*, imparts the characteristic pink color to the cured meat, prevents lipid oxidation providing meat stability (Pegg and Shahidi 2000).

Meat flavor is primarily developed through the cooking process where several reactions take place and many aromas and tastes are generated (Cheng and Ho 1998). The reactions involved are very complex and include the thermal degradation of individual muscle

components, thermal oxidation of lipids, reactions between amino acids and carbohydrates and further interactions between these compounds. The characteristic flavor can not be adscribed to any single compound although it is generally accepted that meaty notes are mainly originated from sulfur-containing compounds generated from water soluble precursors in lean meat (Spanier and others 2004), while fat substances mainly contribute to flavor species differences (Shahidi and others 1986).

Flavor research characterizes and measures overall meat flavor quality and individual attributes by studying the influence of processing conditions. In sensory descriptive methods, the purpose is to identify and measure the presence or intensity of a particular characteristic (Piggott and others 1998). Flavor lexicons constitute a widely used tool for documenting and describing sensory perception. They are used to record and define a product flavor, compare products and determine storage stability, as well as consumer apreciation and acceptability and chemical flavor data (Drake and Civille 2002). The aromatics, tastes and feeling factors are the basis for the lexicon and these lexicons have been used for meat products such as roast beef, frankfurters, bacon, cooked ham, dry-fermented sausages and dry-cured ham as described in this chapter. Sensory descriptive attributes and different evaluation techniques including the methodology (i.e., training, scales, etc.) are summarized at the beginning of each table.

62.3 PROCESSED MEAT PRODUCTS

62.3.1 Roast Beef

Currently, only a small proportion of beef cuts from carcass is used for further processing in comparison to pork and chicken (Boles and Swan 2001). The major reasons for not using beef for further processing are basically related to its the cost and variability of the raw material. So, few different meat cuts may be used in the processing of roast beef. The roast cuts are injected or immersed in a brine containing salt, sugar, polyphosphates, and occasionally, other additives like nitrite, garlic powder, and others. After injection, the roast can be tumbled, vacuum-packaged, and cooked. The sensory characteristics of the roast beef will be developed during the cooking process but the sensory quality will depend on the additives added in the brine. The flavor of raw meat is almost negligible but a desirable flavor is developed during cooking as a result of multiple reactions such as lipid oxidation/degradation and thermal degradation of proteins, peptides, amino acids, sugars, ribonucleotides, and thermal degradation of thiamine (McLeod 1998). In the new preparation procedures of beef roasts several problems have appeared such as warmed-over flavor, reduced juiciness and yield loss due to cooking (Stites and others 1989). The main sensory characteristics studied in roast beef are shown in Table 62.1. One of the main studied characteristics is the warmed-over flavor that consists in an undesirable flavor developed during refrigerated storage of cooked meat due to oxidation of lipids, particularly phospholipids. In this case, the addition of nitrite in the brine contributes to delay the lipid oxidation phenomenon and off-flavor development (Cheng and Ockerman 1998). Other important quality attributes are beef flavor intensity and juiciness and tenderness for texture. For instance, different additives, like polyphosphates, are added during processing affecting the juiciness and tenderness but also acting as an antioxidant although its effect depends on the application of the brine (either surface or injected) (Cheng and Ockerman 1998). Recently, the most important factors underlying beef steak purchase were color, price, visible fat, and cut, while tenderness, flavor, and juiciness were the most related with eating satisfaction

TABLE 62.1 Sensory Descriptive Terms for Evaluating Sensory Characteristics of Roast Beef.

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Trained panel 15 cm anchored unstructured line		Beef flavor intensity Off-flavor intensity		Juiciness Tenderness		Stites and others (1989)
Trained panel Nine point scale	Warmed-over flavor	Warmed-over Roast beef		Juiciness Tenderness		Cheng and Ockerman (1998)
Consumer descriptive sensory panel. Six point structured hedonic scale		Beef flavor intensity Off flavor		Tenderness Toughness Juiciness Dryness	Buying the product Overall acceptability	Boles and Swan (2002)
Consumer descriptive sensory panel. Nine point hedonic scale		Beef flavor	Saltiness	Juiciness Tenderness	Overall acceptability	Robbins and others (2003)
Trained panel		Overall flavor		Tenderness Chewiness Juiciness Binding	Overall acceptability Color acceptability	Lennon and others (2006)

(Robbins and others 2003). In the same study, roast beef produced from cattle supplemented with vitamin E was more acceptable than nonsupplemented controls.

62.3.2 Bacon

Pork bellies are pumped with a curing pickle containing salt, sugar, sodium nitrite, and sodium erythorbate. These pumped bellies are vacuum-tumbled and then, smoked and heat processed. Then, the bellies are chilled, sliced and packaged as an usual format for this product.

The sensory attributes evaluated in bacon are summarized in Table 62.2. In general, characteristic flavor notes of the product like cured pork flavor, smoke, boar, fatty, browned, and others have been evaluated. The four taste attributes and the texture of the bacon have also been described. In this case, texture attributes with importance for the quality of bacon are juiciness and hardness (Berry and Blumer 1981; Sheard and others 2000) as reflected in Table 62.2. However, other evaluated descriptors are crispness and greasiness that are more related with the cooking procedure (frying) applied to the product before consumption (Heymann and others 1996; Maw and others 2001).

The sensory characteristics can be highly affected by processing, addition of different additives (nitrite, salt), and levels of addition, muscle quality, genotype, animal feeding, and sex. The effect of additives on sensory quality of bacon was studied in order to reduce nitrite levels in bacon. The effect of various sodium nitrite and potassium sorbate levels on sensory and chemical properties of bacon was studied (Berry and Blumer 1981). These authors found that the percentage and distribution of lean exert more influence on textural, physical, and cooking properties of bacon than sodium

TABLE 62.2 Sensory Descriptive Terms for Evaluating Sensory Characteristics of Processed Bacon.

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Trained descriptive attribute panel 8-point scale				Hardness Juiciness Total number of chews Mouth coating Fracturability		Berry and Blumer (1981)
Flavor and texture profile panel 13-point scale	Cured pork Fat Briny Sweet Smoke Sour Pig Boar Nose burn Overcooked fat	Cured pork Fat Pig Boar Smoke Old overcooked fat Fatmouth feel Pricklymouth feel	Salt Sweet Sour	Hardness Number of chews Oiliness Crumbliness Fibrousness Coarness of mass		Berry and Blumer (1981)
Trained panel 9-point scale		Bacon Off flavor	Saltiness	Mouth coating Crispness		Heymann and others (1996)
Trained flavor profile panel Magnitude estimation scale with fixed modules (0–15 with reference standards)	Bacon Fatty Smoke Unidentified off Maple	Bacon Fatty Smoke Unidentified off Browned Salt Sour Sweet Bitter	Salt Sweet Sour Bitter			Jeremiah and others (1996)
Trained panel 8-point category scale	Odor Abnormal odor	Flavor Abnormal flavor	Saltiness	Hardness Juiciness	Flavor liking Overall liking	Sheard and others (2000)
Quantitative descriptive Analysis. Trained panel Anchored 10 cm line scale	Boar taint Fishy	Bacon Fatty Salty Fried meat Boar taint Fishy Woody	Salty	Hardness Moistness Greasiness Crispness	*Appearance*: Veined Pinkness Shape	Maw and others (2001)

nitrite and potassium sorbate levels. However, they detected that the partial replacement of nitrite by potassium sorbate results in different chemical-like aromas during frying. They also found that sodium nitrite prevent or delay the onset of strange aromas in vacuum-packaged bacon.

The effect of the feeding system like the increased use of fishmeal in the diet was associated with increased levels of fishy flavor in the bacon (Maw and others 2001). Recently, the addition of n-3 polyunsaturated fatty acids to the feeds in order to improve the fatty acids profile and, thus, the nutritional quality of pig meat, increases the susceptibility of this meat to further oxidation that can be easily detected as off-flavors (Sheard and others 2000).

Animal sex affected the sensory quality of bacon (Heymann and others 1996) as gilts produced less backfat compared to barrows and resulted in bellies that gave a cooked bacon with more protein, less fat, and more cholesterol. The consumer acceptance of bacon is highly dependent on the leanness of pork belly (Heymann and others 1996). This leanness produces better appearance, better palatability, and lower cooking losses. However, Maw and others (2001) did not find differences in bacon between sexes for tenderness, juiciness, saltiness, bacon flavor, and odor although they also detected differences between sexes for abnormal odor and boar odor. The boar taint odor is a urine-like odor being the hormone androstenone one of the major contributors. Those factors that affect androstenone levels such as genotype and environmental factors affect boar taint odor and therefore, the final sensory quality of bacon. Maw and others (2001) also found that boar taint and fishy aromas were competing with the bacon taste. Moreover, these authors also studied the effect of other factors on sensory quality of bacon such as genotype and farming. In this sense, genotype affected the eating quality of bacon specially the saltiness and pinkness. They also found that bacon from straw-court pigs tended to be darker, tender, and more intense in positive flavors than those from slatted and concrete-floored housing. Finally, through the study of the eating characteristics of bacon, Maw and others (2001) suggested a link between intramuscular fat and meat flavor, and between intramuscular fat and texture.

62.3.3 Frankfurters

The standard manufacturing practices in frankfurter processing consist on the preparation of a batter that mostly contains meat (beef, pork, others) and fat, with water, salt, sodium nitrite, sodium ascorbate, polyphosphates, thickeners (starch, milk proteins, others), and spice mix. Initially, meat (beef, pork, others) with half the ice, salt, sodium polyphosphate, sodium nitrite, and sodium ascorbate, are chopped in a vacuum cutter at medium speed. Then, the fat, spice mix, and half the ice, are added to the bowl cutter and mixed. Finally, the thickeners are added and the batter is chopped at high speed to an end-point temperature of around 11°C. Once fully mixed, the batter is stuffed and cooked so that, optionally, can be performed in a smokehouse and vacuum-packaged.

This comminuted meat product is a complex food system where water absorption, gelation, and emulsion formation influence the stability and texture of the product. In addition, the development of the characteristic pink color of this product is due to the formation of nitrosomyoglobin that is stabilized by the cooking treatment. The final structure of the product is achieved during the initial processing stages by the formation of the batter, as a result of coagulation of meat proteins during cooking and gel formation by the added thickeners.

TABLE 62.3 Sensory Descriptive Terms for Evaluating Sensory Characteristics of Frankfurters.

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Trained panel 15 cm unstructured line scale		Flavor intensity Off-flavor intensity	Saltiness	Hardness Cohesiveness Juiciness		Matulis and others (1995a,b)
Trained panel 8-point scale					Coarse (sensory particle size)	Small and others (1995)
Sensory profile Trained panelist, continuous unstructured scale		Meat flavor Smokiness Spiciness Off-flavor Rancidity	Saltiness	Elasticity Juiciness Firmness Greasiness Stickiness Graininess	*Appearance*: Whiteness, Color hue Color strength	Ellekjaer and others (1996)
Consumer panel Untrained panel, 15 cm unstructured line scale		Frankfurter flavor Frankfurter flavor preference		Texture Texture preference	*Appearance*: Color Color preference Overall acceptability	Ho and others (1997)
Consumer panel Untrained panel, descriptive hedonic scales		Flavor intensity Overall flavor Other flavors		Tenderness Juiciness	Overall acceptability	Desmond and Kenny (1998)
Trained panel Hedonic scale		Flavor intensity Overall flavor		Juiciness Overall texture		Lyons and others (1999)
Trained panel Hedonic scale				Hardness Springiness Cohesiveness Gumminess Chewiness Lumpiness Moisture release Overall texture		Cofrades and others (2000)
Untrained panel 9-point hedonic scale		Frankfurter flavor Off-flavor		Texture coarse Juiciness	*Appearance*: Pink color	Yetim and others (2001)

(*Continued*)

TABLE 62.3 *Continued.*

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Trained sensory panel 8-point category scale		Spice flavor Foreign flavor		Juiciness Firmness Springiness Cohesiveness	*Appearance*: Internal color Overall palatability	Yang and others (2001)
Trained sensory panel 9-point hedonic scale				Hardness Juiciness	*Appearance*: Pink color intensity Overall acceptability	Lin and Huang (2003)
Trained sensory panel 10-point intensity scale		Flavor intensity	Saltiness	Firmness Juiciness		Ruusunen and others (2003)
Free-choice profiling Unstructured 10 cm anchored scale	Odor	Smoke Ham	Salty	Juiciness Hardness Coarness	Color	Gonzalez-Viñas and others (2004)
Quantitative descriptive method Trained panel, 9-point unstructured descriptive scale	Odor			Hardness Cohesiveness Springiness		Sabbag and others (2005)
Trained sensory panel 8-point hedonic scale		Flavor intensity Overall flavor		Tenderness Juiciness	Overall acceptability	Hayes and others (2005)
Untrained panel 7-point hedonic scale	Aroma	Chicken flavor		Hardness Juiciness Oiliness	Overall acceptance	Tan and others (2006)

Therefore, the sensory attributes described for frankfurters have been summarized in Table 62.3. Many texture attributes such us hardness, juiciness, cohesiveness, gumminess, chewiness, greasiness, graininess, and others are described in that table. The reason for so many attributes is that all the additives added to the batter can affect them in different ways. The addition of nonmeat proteins (i.e., milk proteins) in these meat products decrease the ingredient cost and increase yield but can affect the sensory quality of the meat product (Ellekjaer and others 1996). Milk proteins act as emulsifiers and therefore, can affect the emulsion formation and thus, the texture. But texture can change from springy at lower protein concentrations to cakey and dry at higher concentrations (Lyons and others 1999). Moreover, these nonmeat protein agents have been added to reduce the fat content of sausages and improve its unhealthy image for the high fat levels (20–50%). However, the reduction of the fat content in final comminuted meat products to less than 20% can produce unacceptable texture, flavor, and appearance. For example, the overall palatability of low-fat frankfurters has been directly related to firmness and inversely related to juiciness and spice and foreign flavor of the product (Yang and others 2001). Soy protein concentrates and isolates have also been used successfully to reduce the fat content in sausages (Ho and others 1997) although they should be added to frankfurters at $<3.0\%$ in order to avoid an increased hardness and off-flavor and decreased juiciness, saltiness, and flavor intensity (Matulis and others 1995a). Taking into account that the reduction of fat in a meat emulsion product is generally accompanied by an increase in added water, this procedure will only be effective if this water is retained after thermal processing, cooling, and packaging. Therefore, starches and gums are being used as fat substitutes because they can maintain the functional properties of the product while imparting fat-like properties. Carragenan and oat fibre can improved the juiciness, cohesiveness, and lumpiness of low-fat sausages but none of them produce the same sensory attributes as the full-fat sausages (Cofrades and others 2000). Blends composed of carrageenan in combination with tapioca starch improve the final texture of low-fat sausages (Lyons and others 1999).

On the other hand, the manufacture of sausages with a reduced amount of salt is more difficult than expected and may cause sensory changes. So, the reduction in salt content decreases protein extraction and water binding in addition to altering palatability attributes. The lower ionic strength produces increased release of fat and gel liquid although it will depend on the degree of salt reduction (Matulis and others 1995b). Finally, processing conditions may affect sensory properties of frankfurters. The chopping time and final batter temperature of the comminuted product have a significant effect on the stability of meat batter. The mixing develops a uniform distribution of fat and lean particles but the changes in particle size affects sensory and physical characteristics more than changes in mixing time. In the case of additional mixing, a decrease in the particle size and increase of springiness and chewiness has been reported in cooked frankfurters (Small and others 1995).

On the other hand, flavor sensory attributes typically described in frankfurters are flavor intensity, frankfurter flavor, off-flavor, and spice flavor. However, their flavor attributes will depend on the additives added to the meat emulsion like spices and liquid smoke. Many compounds that contribute to the overall odor of frankfurters have been identified, being the smokiness and spiciness of frankfurters caused by phenols, terpenes, and other compounds such as aldehydes, ketones, furanthiols, and alicyclic sulfur compounds, derived from the meat fraction (Chevance and Farmer 1999). Saltiness is the most important taste attribute (Matullis and others 1995b; Ellekjaer and others 1996; Runsumen and

others 2003). Finally, other descriptors are color, appearance, and overall acceptability. In recent years, quality evaluations of commercial frankfurters have been done in order to evaluate consumer preferences (González-Viñas and others 2004) and to correlate physicochemical parameters with deterioration indexes (Sabbag and others 2005).

62.3.4 Cooked Ham

The main purpose in the manufacture of cooked ham is to develop the characteristic flavor and color, as well as an appropriate texture that allows the product to be sliced. Therefore, during processing the pork legs are cleaned from skin, bones, connective and adipose tissues, and also zones that can be nonattractive to consumers. Then, the hams are injected with a brine containing salt, sugar, nitrate or nitrite, ascorbate or erythorbate, aromas, and in some cases, polyphosphates and other authorized additives. Once injected, hams are vacuum-tumbled to allow the brine to penetrate the full piece. The typical nitrosmyoglobin cured color is developed and stabilized by cooking. The salt and phosphates contribute to the solubilization of proteins and the cohesion of the product which is favored under vacuum, especially with further cooking. After tumbling, the meat is vacuum-packaged in heat shrinkable cooking bags or canned, to be cooked at a final core temperature of around 67–71°C or higher, depending on the composition. This process stabilizes the product, from the microbial point of view, and its color, produces the characteristic texture of the ham by protein coagulation and finally, develops the characteristic flavor. This cooking process allows the ham to be sliced while keeping its structure.

There are many varieties of ham products made from pork leg or shoulder including traditional, modern tumbled, and canned. However, its sensory properties in relation to acceptability are not well defined (Delahunty and others 1997). In cooked hams, the sensory attributes related to appearance, texture, and flavor should be considered as can be observed in Table 62.4 where a high number of attributes are shown. Several studies have been done to compare traditional hams with those produced through modern technology. The evaluation of a large variety of hams (traditional, modern tumbled, and canned) by descriptive methods indicated that the better textured hams were not plastic, were flaky and fibrous with a stronger ham flavor, and without bitterness (Nute and others 1987). The hams with poorer appearance, texture, and flavor were those juicier and with a wetter appearance. In summary, the hams that were meaty, nongelatinous and lean were scored for a better appearance. The quality of the hams is influenced by the choice of meat cut, composition, and quantity of the brine injected, rate and extent of tumbling or massaging, and the cooking time and temperature. The effect of additives like sodium chloride or sodium nitrite on the sensory attributes of ham have been studied. Sodium chloride was related to saltiness and nitrite was positively related with the intensities of the appropriate sweet taste and aftertaste and negatively related to the inappropriate sour taste (Jeremiah and others 1996). Another study focused on the effect of intramuscular fat content on the sensory quality of cooked ham because it is generally accepted that intramuscular fat content has a positive influence on the sensory quality of pig meat. The intramuscular fat content did not significantly affect the sensory attributes of cooked hams except the perception of marbling (Fernandez and others 2000). Due to the effects that meat quality and processing conditions produce on sensory quality of cooked ham, many works have been done to study the effect of preslaughter treatment (Fernandez and others 2002), different cooking and cooling methods (Desmond and others 2000, 2002; Cheng and others 2005), and injection levels (Desmond and others 2002). The

TABLE 62.4 Sensory Descriptive Terms for Evaluating Sensory Characteristics of Cooked Ham.

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Sensory profile analysis 10 cm unstructured lines		Ham flavor Bacon flavor Porky flavor Spicy Smokiness Overall flavor (hedonic)	Saltiness Sweetness Bitterness	Rubberness Flakiness Firmness Plastic Cohesiveness Fibrous Juiciness Tenderness Overall texture (hedonic)	*Appearance*: Fatness Uniformity of lean Dominant color Wetness Plastic Gelatinous Overall appearance (hedonic)	Nute and others (1987)
Trained panel 10-point scale	Odor Off-odor Urine-like odor	Flavor Off-flavor Urine-like off-flavor Acid off-flavor	Brine taste	Tenderness Juiciness Doughy Fibrous	*Appearance*: Pink color Homogeneity of color Slice wetness Slice steadiness Overall opinion	Bonneau and others (1992)
Trained flavor profile panel Magnitude estimation scale with fixed modules (0–15 with reference standards)	Ham cure Smoke Fatty Unidentified off Metallic Salt	Ham cure Smoke Fatty Unidentified off Metallic Salt Sweet Sour Bitter	Sweet Sour Salt Bitter			Jeremiah and others (1996)
Free-choice profiling Untrained consumers 10 cm anchored scale		Flavorful Meaty Intensity Processed Smoked Hammy	Salty Sweet Sour	Chewy Tough Moist Rubbery Crumbly Firm	*Appearance*: Dark color Moist Color uniformity Marbling Processed	Delahunty and others (1997)

(*Continued*)

TABLE 62.4 *Continued.*

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
		Salty Sweet Sour		Strong structured Processed Fibrous	Rough Shiny Plastic Rubbery	Delahunty and others (1997)
Trained panel Descriptive hedonic scales, 6 points		Overall flavor	Saltiness	Tenderness Juiciness Overall texture	*Appearance*: Cured color Overall acceptability	Desmond and others (2000, 2002)
Trained panel 7-point intensity scale		Smell Flavor		Dryness Toughness	*Appearance*: Color Marbling	Fernandez and others (2000)
Trained panel 7-point intensity scale	Odor	Flavor	Salty	Dryness Toughness Smoothness Stringiness	*Appearance*: Color Color homogeneity Marbling Slice cohesiveness	Fernandez and others (2002)
Trained panel Descriptive hedonic scales, 6 points		Flavor		Tenderness Juiciness Binding	*Appearance*: Color Overall acceptability	Cheng and others (2005)

different cooling methods applied during the processing of cooked ham affect the tenderness, juiciness, overall texture and acceptability, and the cured color (Desmond and others 2000). The halothane genotype of pigs can also affect the sensory quality of cooked ham affecting mainly the textural properties. So, cooked hams produced from nn pigs were drier, tougher, stringier, and less smooth than those produced from NN and Nn pigs (Fernandez and others 2002).

On the other hand, the effect of male sex hormone (androstenone) present in the muscle of noncastrated pigs and skatole on the sensory characteristics of cooked ham have also been studied obtaining that these compounds affect negatively the sensory attributes of odor and overall opinion. Attributes such as urine-like off-odor, flavor, off-flavor, urine-like off-flavor, and acid-like off-flavor have been described (Bonneau and others 1992).

The development of the characteristic cooked ham flavor depends on the cooking process through thermal reactions as already explained for bacon as well as any aromas added in the brine. However, no compound has been clearly identified yet for cured aroma in cooked ham (Guillard and others 1997).

62.3.5 Dry-Fermented Sausage

There is a wide variety of fermented sausages depending on the raw materials and processing conditions. Briefly, minced meat and additives are mixed and stuffed into casings. Then, sausages are placed in curing chambers for microbial growth during fermentation that usually takes 1–2 days and afterwards to ripening/drying that ranges between 7–90 days. The length of the process depends on many factors although the most important are the type of product, its diameter, drying conditions and desired flavor development. In general, sausages tend to be more humid and less flavored when they are short ripened.

In the case of texture, tenderness/hardness and juiciness are the most common evaluated descriptors (Berry and others 1979; Roncalés and others 1991; Hoz and others 2004; Moretti and others 2004; Pérez-Cacho and others 2005). The texture of sausages is initially affected by the acidification during the fermentation stage and afterwards by dehydration during drying/ripening. As a result of lactic acid accumulation, pH approaches the isoelectric point of proteins. Once pH is reached, proteins coagulate and reduce the water retention capacity that contributes to firmness (Talon and others 2002). Textural characteristics of the sausage like firmness, hardness, and cohesiveness of meat particles change during drying and are usually correlated to time, moisture content, sausage diameter, and the initial grinding size (Acton 1977). Dry-fermented sausages are usually sliced for consumption and this means they need a good consistency and as homogeneous as possible. Sometimes, drying is excessive and sausages tend to be softer in the center and harder in the rind (Toldrá 2002).

Color is mainly due to the reaction of nitrite with myoglobin producing the red color (Pegg and Shahidi 2000). Essentially, the nitrite initially added to the sausage is reduced to nitric oxide either enzymatically or chemically (favored by ascorbic/erythorbic acid). Then, myoglobin and nitric oxide interact forming red-colored nitric oxide myoglobin (Demeyer and Toldrá 2004). Sometimes, the color is not homogeneous through the entire slice due to moisture gradient into the sausage as a result of a noncorrect drying process (Pérez-Alvarez and others 1999). In addition to the color intensity, other descriptors are related to the appearance like particle size and fat content (Beilken and others 1990), visual cut appearance, total fat and external mould cover (Roncalés and others 1991).

The pH drop during carbohydrate fermentation is essential for the sensory characteristics of the sausages, especially taste. So, those sausages rapidly processed with an intense lactic acid generation and a deep pH drop below 4.8, exhibit a predominant sour taste and a poor aroma. Italian Milano salami is prefered when it is medium aged, having high pH values and low amount of lactic acid although the preference is reduced when product is excessively ripened and with a high salt/moisture ratio (Casiraghi and others 1996). In addition to sourness, saltiness is also oftenly evaluated because fermented sausages may contain between 2–3% of salt (Benito and others 2004; Moretti and others 2004).

Aroma descriptors are quite varied, as reflected in Table 62.5, and usually include terms like hot spice, smoke, rancid, butter, and fat. The aroma of the sausage depends on the generation of volatile compounds but this profile changes according to the specific starter culture used for fermentation, typically *Staphylococcus* and/or *Micrococcus*. These starters contribute to lipolysis and the metabolism of the released fatty acids as well as nitrate reduction to nitrite (Montel and others 1993; Toldrá and others 2001; Stahnke 2002). In this way, different aroma compounds may be found. For instance, the addition of *S. saprophyticus* and *S. warneri* contributes to the odor of butter which is correlated with acetoin, diacetyl, 1,3-butanediol, and 2,3-butanediol (Berdagué and others 1993a). 2-Pentanone, 2-hexanone, and 2-heptanone, correlated with curing odor, are obtained with the addition of *S. carnosus* + *P. acidilactici*, *S. carnosus* + *L. sakei*, *S. carnosus* + *P. pentosaceus*, and the addition of *S. saprophyticus* is related with lower rancidity.

The spices and condiments are typically added to dry and semi-dry-fermented sausages and have a strong contribution to the aroma profile of the product. So, it is relatively usual to find either pepper, paprika, mustard, oregano, rosemary, garlic, or onion (Ordoñez and others 1999). Depending on the added spice, different volatile compounds can be found. So, some terpenes like α-pinene, β-pinene, β-mircene, e-carene, and limonene are usually present in sausages produced with pepper while several sulfur compounds may appear when garlic was added (García-Regueiro and others 1998).

The improvement of the sensory properties of dry-fermented sausages has been recently studied by the addition of free amino acids (Herranz and others 2005) or through the addition of bacterial extracts that enhance the amino acid breakdown (Herranz and others 2006).

62.3.6 Dry-Cured Ham

Dry-cured ham is a typical product produced in certain areas of Europe, China, and United States. The process is generally very long, between 3 to 24 months, and depends on the type of product and desired quality. Main stages consists in salting, where the salt (salt with nitrate and nitrite) is rubbed onto the surface of the hams and maintained at refrigeration temperatures during several days for salt penetration, postsalting, where the hams are cleaned and left at refrigeration temperatures for salt diffusion, and finally, the ripening/drying stage where different temperatures and relative humidity are applied for drying and enzymatic development of flavor.

The development of the sensory characteristics of dry-cured hams is due to the action of the curing agents (salt, nitrite, or nitrate) and processing conditions. In this case, the stabilization of the ham is due to the dehydration of the product. The development of the characteristic color is due to the interaction of nitric oxide with myoglobin as explained before. In this type of product, cured flavor is developed through a complex mechanism that

TABLE 62.5 Sensory Descriptive Terms for Evaluating Sensory Characteristics of Dry-Fermented Sausages.

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Trained sensory panel		Flavor intensity Undesirable flavor		Tenderness Juiciness		Berry and others (1979)
Untrained panel 8-point hedonic scale		Flavor		Tenderness Juiciness	*Appearance*: Color Overall satisfaction	Berry and others (1979)
Free choice profiling (selection) Fixed choice profile Unstructured scale with end points defined by consensus	Acid/sour Off-aroma Smoke Acceptability of aroma	Acidic/sour Hot/spice Off-flavor Smoke Acceptability of flavor		Initial bite/adhesion Chewiness Greasiness Acceptability of texture	*Appearance*: Color Particle size Fat content Acceptability of appearance Overall acceptability	Beilken and others (1990)
Semitrained assesors 9-point intensity scales	External smell intensity External smell quality Smell intensity Smell quality	Flavor intensity Flavor quality		Toughness Juiciness Overall mouth perception	*Appearance*: Visual external Mould cover Resistance to pressure Overall external perception Visual cut appearance Total fat Ease of skin peeling Color Overall cut perception Overall acceptability	Roncales and others (1991)
Quantitative descriptive method Trained panel, unstructured line scale of 15 cm	Odor intensity Salami Cheese Sourdough Sourish Fatty Rancid Nauseous Burned Solvent					Stahnke (1995)

(*Continued*)

TABLE 62.5 *Continued.*

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Trained panel Nonstructured scales		Dry-cured ham flavor Dry-cured sausage flavor Vinegar flavor Nutty flavor Milk flavor Butter flavor Pate flavor Fat flavor				Rousset-Akrim and others (1997)
Trained panel Nonstructured 10 cm hedonic scale		Odor	Taste	Texture	Color Overall quality	Bruna and others (2000, 2001) Selgas and others (2003) Herranz and others (2005, 2006)
Trained panel Nonstructured 10 cm hedonic scale	Odor		Taste Sweet Bitter Sour Salty Meat impression	Texture Juiciness	Color	Hoz and others (2004)
Quantitative descriptive analysis		Aroma intensity Cured aroma Rancid	Saltiness Sweetness Bitterness Acidness	Hardness Softness Fibrousness Juiciness	After taste	Benito and others (2004)
Semitrained panel 6-point intensity scale		Rancidity	Saltiness Acidity	Hardness Elasticity Cohesiveness	Color intensity Overall acceptability	Moretti and others (2004)
Descriptive sensory panel	Black pepper Lactic acid Mould Spice odor	Black pepper Mould Spice aroma	Acid Salty	Hardness Juiciness	*Appearance*: Exudate Fat/lean connection Luminance Presence of crust	Perez-Cacho and others (2005)
Quantitative descriptive analysis	Typical sausage odor		Fishy taste	Juiciness	Color intensity General acceptability	Valencia and others (2006)

TABLE 62.6 Sensory Descriptive Terms for Evaluating Sensory Characteristics of Dry-Cured Ham.

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
Trained panel, unmarked anchored line scales Dry-cured ham (Country style ham) broiled		Country ham flavor Off-flavors	Saltiness	Tenderness		Harmon and others (1992)
Trained panel Continuous scale from 0 to 100 French dry-cured ham	Ham Raw meat Fat Meaty Rancid Persistence of ham		Salty Piquant			Berdague and others (1993)
Quantitative descriptive analysis Scale from 0 to 5 Italian Parma ham	Fresh pork Aged		Aged Salty Acid Bitter Butter			Careri and others (1993)
Trained panel Graduate scale of 10 cm French dry-cured ham		Dry ham Fresh meat Fat Cured Rancid Metal	Salty Acid	Firmness Dryness Fibrousness Mellowness	*Persistence of aromas*: Dry ham Metallic Taste after deglutition Astringency	Buscailhon and others (1994)
Quantitative descriptive analysis Unstructured intensity scale from 1 to 15 Italian Parma ham		Nutty Cheesy Meaty Stale Fatty	Salt			Hinrichsen and Pedersen (1995)
Quantitative descriptive analysis Serrano dry-cured ham		Fat complex Boar-taint Barnyard Haylike/musty Brown spice Pickling spice Smoky	Salty Sour Bitter		*Appearance*: Marbling Color homogeneity Tyrosine crystals *After-taste*: Astringent Metallic	Flores and others (1997)

(*Continued*)

TABLE 62.6 *Continued.*

Technique	Aroma	Flavor	Taste	Texture	Others	Reference
		Pork Serum Pungent Nutty Rancid			Mouthfilling (umami)	
Quantitative descriptive analysis Unstructured 10 cm scale Iberian dry-cured ham	Aroma intensity Acorn ham aroma	Flavor intensity Aftertaste Cured Rancid	Saltiness Sweetness Bitterness	Fat firmness Hardness Dryness Fibrousness Juiciness	*Appearance*: Yellowness of fat Pinkness of fat Oiliness of fat Redness of lean Bright of lean Marbling of lean	Ruiz and others (1998, 2002)
Sensory textural properties Trained panel, nonstructured quantitative scale Serrano dry-cured ham				Hardness Pastiness Crumbliness Adhesivity		Guerrero and others (1999)
Quantitative descriptive analysis Serrano dry-cured ham		Fat complex Barnyard Pickling spice Pork Serum Nutty Rancid	Salty Sour Bitter		*Appearance*: Marbling Color homogeneity Tyrosine crystals Overall quality *After-taste*: Astringent Mouthfilling (umami)	Armero and others (1999)

Trained panel Seven point intensity scale French dry-cured ham		Odor intensity Flavor intensity	Salty	Dryness Smoothness Toughness Fondant	*Appearance*: Cohesiveness between muscles Subcutaneous fat depth Yellow color of fat Marbling Muscle red color intensity Muscle color homogeneity Areas of abnormal color	Fernandez and others (2002)
Quantitative descriptive analysis Unstructured 10 cm scale Iberian dry-cured ham	Odor intensity Rancidity Cured	Flavor intensity Cured Rancid After-taste Toasted Non-pleasant	Saltiness Sweetness Bitterness	Firmness of lean Dryness Fibrousness Juiciness Pastiness	*Appearance*: Redness of lean Brightness of lean Marbling of lean	Andres and others (2004)
Untrained consumer panel 9-point hedonic scale		Flavor intensity	Salty	Hardness	Overall acceptance Appearance	Flores and others (2006)
Trained panel, structured 9-point scale	Dry-cured ham odor	Cured ham flavor Rancid flavor	Saltiness	Hardness Crumbliness Pastiness Fibrousness Adhesiveness	*Appearance*: Intramuscular fat Fat thickness Cured color Color homogeneity	Cilla and others (2006)

generates flavor precursors through proteolysis and lipolysis (Toldrá and Flores 1998). In the last years, there are many studies of dry-cured ham aroma where 100 of volatile compounds were isolated and identified including their contribution to specific aromas (Flores and others 1998).

The sensory quality of dry-cured hams is highly affected by premortem factors (genetics, animal species, sex, etc.) meat quality, curing salt composition, and rate and extent of the drying process. Many different sensory attributes have been described in dry-cured ham and are summarized in Table 62.6. The feeding system of pigs can affect the sensory quality because when pigs are raised in a free-range system, give processed hams with better scores in oiliness, brightness of the lean, marbling, aroma, and flavor traits (Cava and others 2000). The effect of feed is quite pronounced and mainly affects the lipid content and fatty acids composition. In general, hams from females and Duroc-sired pigs give a better overall quality than those hams from Belgian Landrace-sired pigs characterized by a poor quality and rancid aromas (Armero and others 1999). Also, Duroc sire lines are more related to higher intramuscular and subcutaneous fat sensory perception, as well as lower sensory adhesiveness (Cilla and others 2006). The influence of meat quality on sensory attributes has been demonstrated by the effect of meat pH on sensory textural attributes, as it is the case of DFD hams (high pH meat) that give softer, pastier, more crumbly and adhesive dry cured hams than normal pH meat (Guerrero and others 1999).

The effect of salt content and processing conditions has been studied in Iberian dry-cured ham where the addition of lower salt content produced dry-cured hams less salty but with softer textures which could affect the overall quality of the hams (Andres and others 2004). These authors also studied the effect of modified processing conditions for dry-cured Iberian hams using lower temperatures during the drying stage but they lead to more rancid aromas. The length of the process constitutes another important factor of variation in quality attributes, especially in the aroma and taste traits of ham. The reason is that proteolysis and lipolysis are highly affect by the conditions of ripening/drying. Therefore, the generation of free amino acids is somehow correlated with the length of the process. This is the case found for glutamic acid, aspartic acid, methionine, isoleucine, leucine, and lysine (Flores and others 1997a; Toldrá and others 2000). Some of the generated free amino acids are also related to flavor. This is the case of lysine and tyrosine that are related with a better quality of aged taste of hams while asparragine is related to worse quality. Glutamic acid contributes to salty taste and phenylalanine and isoleucine to sour taste (Careri and others 1993). However, the sensory characteristics are highly affected by the origin of the product. For instance, little effect has been found of amino acids on the development of the aroma of cured meat in French-type ham (Buscailhon and others 1994). The effect of the length of the process is also important in the development of the cured aroma like in the case of Parma hams ripened for 12 months where an increase of several compounds was detected (3-methyl butanal, ethyl esters, and alcohols like propanol, 1-butoxy-2-propanol, and 2-butanol) (Bolzoni and others 1996). These hams contain a higher proportion of esters because nitrate is not added with the curing salt. Other compounds like 2-pentanone, 1-pentanol, ethanol, ethyl acetate, 1-penten-3-ol, and pentanal have been found to increase in French hams (Buscailhon and others 1993). The length of the process affects primary Iberian ham flavor while texture and appearance is not affected when comparing short and long processing times (Ruiz and others 1998) at the same time, slice location affects the texture attributes but it produces few differences in aroma and flavor attributes.

Iberian and Corsican hams are characterized by very long processing times, usually 24 or even more months, and exhibit a high intramuscular fat content due to genetics. Therefore, attributes such as yellowness of fat, pinkness and oiliness of fat are important to the sensory quality. These hams contain a higher amount of methyl ketones, aldehydes, branched aldehydes, and alcohols which are positively correlated with odor and flavor traits like flavor strength, cured flavor, and after taste (Ruiz and others 1998) and negatively for rancidity (Ruiz and others 1999). Other long processed hams, like Serrano hams, give scores with higher barnyard, sour and salty taste (Flores and others 1998b). Country-style hams and other short processed hams are characterized by a lower content in volatile compounds and a characteristic fresh-cured pork flavor. Regarding texture, the external muscle *Semimembranosus* tends to be harder and dryer than the internal *Biceps femoris* that tends to be softer and more humid (Cava and others 2000). Typical descriptors are hardness, dryness, fibrousness, and juiciness (Buscailhon and others 1994; Ruiz and others 1998, 2002; Guerrero and others 1999). The acceptability of Iberian dry-cured ham is highly dependent on juiciness and flavor intensity; therefore, the raw material and processing conditions are the main factors that influence acceptability, since juiciness is mainly based on intramuscular fat content which depends on raw material composition, and flavor is developed during processing (Ruiz and others 2002).

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Section XIV

Milk and Milk Products

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

63

Processing Quality Fluid Milk Products

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63.1 Introduction 332
63.2 Quality Begins at the Milk Source: The Dairy Cow 332
63.2.1 Defining Raw Milk Quality 332
63.2.2 Farm Bulk Tank Operation 334
63.3 Entering the Processing Environment: The Dairy Plant 334
63.3.1 Receiving the Raw Milk at the Dairy Processing Plant 334
63.3.2 The Processing Environment 335
63.3.2.1 The Pasteurization Process System 335
63.4 The Pasteurization Process 336
63.4.1 The Process Parameters 336
63.4.2 Standardizing the Milk Fat Content (Gösta 2000) 337
63.4.3 Minimizing Postpasteurization Contamination 337
63.4.4 Filling Operations and the Market Milks Produced 338
63.4.5 High-Temperature-Short-Time (HTST) Pasteurized Fluid Milk Products 338
63.4.6 Maintaining the Temperature of the Finished Product Below 4°C (40°F) 338
63.4.7 In-Container Sterilization 338
63.5 Aseptic Processing and Packaging of Fluid Milk (Extended Shelf-Life Milk) 339
63.5.1 The Concept 339
63.5.2 Aseptic Processing and Packaging: The System 339
63.5.3 Equipment Design and Operation 339
63.5.4 Packaging Platforms 342
63.5.5 Milk System Stability Considerations and Product Shelf Life 342

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

63.6 Written Standard Operating Procedures 343
63.6.1 Employee Training 343
63.6.2 Compliance with Public Health Regulations 344
63.7 A Final Comment 345
References 345

63.1 INTRODUCTION

"Fresh" is a frequent term used to describe food "quality." And "fresh" and safe milk products delivered to the customer becomes the goals of all dairy processors. It is to this end that the U.S. dairy industry has fine-tuned its milk collection, storage, and handling practices through a cooperative effort between the dairy cooperatives, milk processors, the State health agencies, and academia. Results of those efforts culminate in a published "model" guideline referred to as the "Grade A Pasteurized Milk Ordinance (PMO)" published by the U.S. Department of Health and Human Services Public Health Services, Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD. Much of what is discussed in this chapter is supported by that publication.

63.2 QUALITY BEGINS AT THE MILK SOURCE: THE DAIRY COW (Chambers 2002)

Paramount to producing quality, safe milk is the health state of the individual dairy cow. With today's trend toward larger dairy herds (cow numbers exceeding 1000), biosecurity of the dairy herd becomes important. Therefore, as new cows are imported into the milking units, they are placed in quarantine for a period of time to assess the health state of the dairy animal and milk production. Once the cow passes its quarantine, it is then introduced into the main milking herd.

Milking units are individual farm complexes where the dairy cows are housed and fed, breed, have calves, and are milked. These facilities provide a clean, efficient production environment for the production of large quantities of quality milk ready for delivery to a milk processing plant.

63.2.1 Defining Raw Milk Quality

Defined measurements of raw milk quality are the following Grade A Standards (USHHS) (Table 63.1). For details of the methodology for the above analyses, the reader is referred to the *Standard Methods for the Examination of Dairy Products* published by the American Public Health Association, Washington, DC.

TABLE 63.1 Grade A Standard Raw Milk.

Temperature	7°C (45°F)
Bacterial limits	100,000/mL
Antibiotics	No positive results
Somatic cell count, per mL	750,000 cows 1,000,000 goats
Sediment	Grade 2
Added water	Freeze point −0.525°C

The temperature standard refers to that temperature of the raw milk as stored in the farm bulk tank. The PMO requires that freshly collected milk must be brought to 7°C (45°F) (Table 63.1) within 2 h after collection and maintained at that temperature or below prior to pick-up. Higher temperatures could result in bacterial growth and biochemical activity with off odors and flavors developing. Milk safety issues might also develop such as the growth of *Staphylococcus aureus* and the production of an enterotoxin. This toxin is quite heat stable and could remain active after a pasteurization treatment.

The 100,000/mL bacterial limit reflects the cows' udder health, milk handling environment, and the cleanliness of the milking equipment. Usually, when high bacterial numbers are encountered, the milk contact surface areas of the milk-handling equipment become prime suspect for not being properly cleaned and sanitized. For example, if the caustic-chlorine cleaner is at an inadequate concentration or the cleaning solution temperature is lower than prescribed, its cleaning power becomes substantially weaker and milk soil may not be sufficiently removed. When this soil is contaminated with untreated well water, one has a condition to promote bacterial growth and high numbers of bacteria. When freshly collected raw milk passes over these bacteria-contaminated surfaces, the bacterial numbers go up in the milk prior to entering the farm bulk storage tank.

No antibiotic residues are permitted in Grade A raw milk. The presence of antibiotics in milk should be prevented with sound animal handling practices. That is, antibiotic treated cows should be **boldly** marked so that these cows can be milked separately and the milk collected does not enter commercial channels. It is a serious quality offense that is addressed by the regulatory agencies when antibiotic contaminated milk is detected in these channels. A dairy producer can receive severe financial penalties as well as lose their Grade A permit should he/she violate the antibiotic residue prohibition regulation three times within a 12-month calendar year. The underlying principles behind this prohibition are that low antibiotic residues promote consumer allergic reactions and resistance for disease producing bacteria.

As previously stated, sound cow health is essential to a quality milk source. While there are several health conditions that can develop in a dairy cow, the most common one is mastitis. Mastitis is caused by a multiple of infectious agents that can damage the milk producing cells in the udder thus, lowering the cow's milk synthesizing capacity. During the microbial infection process, the cow's defense system is stimulated and one response is the production of somatic cells (many are white blood cells) to isolate and fight off the infecting agent. So the Somatic Cell Count is used to assess the health condition of the cow's udder. Counts above 750,000 for cows usually can be correlated with the presence of udder infections within the milking herd. When the farm bulk tank milk samples demonstrate Somatic Cell Counts above this number, it becomes prudent to screen the cows of that milking unit for mastitis infections.

The Sediment Grade Standards, developed by the U.S. Department of Agriculture, evaluate the level of physical cleanliness of the raw milk at the farm level. This Standard is incorporated into the State health agency's farm inspection protocol, as well as, the milk producer's dairy cooperative's oversight. During the farm inspection process, samples are routinely taken from the bottom of the farm bulk tank and passed through a standardized filter pad. The test pad is compared to sediment pad standards to determine its assigned grade. Grade assignments above 2 indicate that poor cleaning of the cows' teats and udder are occurring. A higher sediment grade above 2 reflects that excessive soil and manure have entered the milk during the collection procedure.

Added water is an economic issue between the buyer (the dairy processor) and the seller (the milk producer). Two major factors enter into the enforcement of the added water standard. The first is the recognition that some incidental water will enter the milk during rinsing operations. General agreement of enforcement allows no more than 2% added water with a freezing point of -0.525°C. Higher freezing point values translate into an "added water" violation. The second factor is that raw milk is paid for based on its weight. Thus, by adding water to the bulk tank and blending it in with the milk increases the volume translation into weight that skews the economics in favor of the milk producer. Added water dilutes the milk solids and thereby reduces the dairy products, such as Cottage cheese, derived from that milk source.

63.2.2 Farm Bulk Tank Operation (Gösta 2000; Chambers 2002)

The farm bulk tank has two principle roles to play. The first is to collect and store the daily milk harvested. The second is to cool the raw milk from 35°C (95°F) down to 7°C (45°F) within 2 h after the milk enters the bulk tank. The bulk tank's cooling medium may be either a direct-expansion or ice bank system. The direct-expansion is a more efficient heat transfer system than the ice bank. The dairy farms operating under the Grade A PMO must deliver their raw milk to the dairy processing plant within 48 h after its collection. With the larger dairy cow milking units, sufficient quantities of milk are produced within 24 h that the milk, in specific cases, could fill an over-the-road tanker, on average, every 4 h and be shipped to commercial channels.

It should be noted that the farm bulk tank could be a source for milk quality problems. One problem lies within the failure of the dairy producer to turn on the refrigeration unit when newly collected milk enters the tank. The heat from the incoming milk either is not removed, thus maintaining ideal bacterial growth conditions and initiates lipase activity, or elevates the temperature of the previously cooled milk to activate microbial and enzymatic activity. Whether microbial or enzymatic activity does occur, in either case, flavor change can take place to the point that the raw milk could be unsaleable. Excessive agitation can result in a "churning" effect indicated by chunks of cream floating in the milk. A third common problem comes from a too efficient cooling of the milk resulting in the masking of potential sanitary problems with the tank itself. This latter problem is addressed by use of the Preliminary Incubation Count procedure when assessing the sanitary conditions of the farm bulk tank.

63.3 ENTERING THE PROCESSING ENVIRONMENT: THE DAIRY PLANT (Gösta 2000; Boor and Murphy 2002; Chambers 2002)

63.3.1 Receiving the Raw Milk at the Dairy Processing Plant

Depending on the distance of the raw milk source, time in transit may range from one hour up to as much as 72 h. Since the milk remains in the raw state, maintaining a temperature at 7°C (45°F) or below is critical to minimizing biological and chemical change.

At the dairy plant, the milk is checked for temperature, acidity, antibiotics, and added water. Failure to meet the previously defined Standards could result in rejection of that milk load. When this load is found acceptable, it is downloaded and passed through a plate cooler to reduce the temperature to 4°C (40°F) on its way to the silo storage tanks.

63.3.2 The Processing Environment

As raw milk is processed into a variety of fluid milk products, four overriding factors dictate the management of the processing environment. These are: the pasteurization process; standardizing the milk fat content; minimizing post-pasteurization contamination; and, maintaining the temperature of the finished product below 4°C (40°F).

63.3.2.1 The Pasteurization Process System (USHHS 1999 Revision; Gösta 2000). The pasteurization process system is well defined in the PMO. Because the PMO is given statutory law status in the United States by its adoption by the individual State legislatures, strict adherence to the PMO requirements must be followed. Item 16p of the PMO specifically states "Pasteurization shall be performed as defined in Section I, Definition V of this (PMO) *Ordinance.* Aseptic processing shall be performed in accordance with 21 *Code of Federal Regulations* Parts 113 [and] 108, and the Administrative Procedures of Item 16p, C, D, and E of this section."

The focus of the PMO requirements is to document critical control points relevant to time–temperature control in the pasteurization process. In addition, by system design, a means of diverting the under-pasteurized milk process stream back to a balance tank and incorporating a higher-pressure gradient on the heat exchanger plates are required. The latter is to prevent unpasteurized milk from short circuiting to the pasteurized side of the heat exchanger plates should "pin-holes" develop in the plate.

The *system components* of the commonly used high-temperature-short-time continuous-flow pasteurizer are the three heat exchangers, the timing pump, and the holding tube.

The *pasteurizer's heat transfer components* consist of three sections: heater, regeneration or water removal, and cooling. There are two basic methods of transferring heat into milk. The most common method is by indirect heat transfer through a physical barrier. The second method is by direct transfer of heat via steam infusion/injection.

Of the indirect methods used, the plate heat exchanger is most commonly used by the dairy industry. Depending on the targeted temperature range one is operating, hot water is the common medium used for putting heat into the milk (heater section) and, the incoming cold, raw milk (regeneration section) and chilled water (cooling section) are used to remove heat from the milk.

With respect to the use of the direct heating of milk, one must be sure to use only culinary steam for this purpose. Two approaches are used by the dairy industry, one is steam injection and the other is steam infusion (falling film). In both applications, water must be removed from the milk by evaporation, thus requiring an additional processing step and equipment. The more common applications for direct heating are in the heating of cultured cheese whey (steam injection is used) and pasteurizing ice cream and soft serve mixes (falling film is used).

The *timing pump* may either be a variable controlled positive displacement pump or a homogenizer. In either case, the appropriate flow rate is established and pressure is applied to the pasteurizer system. Whether a positive displacement pump or homogenizer is used, in either case, the pumping rate must be documented and the pump speed "sealed" by the State health agency for the established flow rate. The location in the system is after the regeneration section of the plate heat exchanger. A booster pump is used in front of the timing pump to maintain a milk flow in the pipeline and the raw side of the regeneration section. This pump is of centrifugal design and does not influence the milk flow

characteristics after the timing pump. Thus, the necessary 6.9 kPa (1 psi) pressure differential is established between the pasteurized milk and the raw milk across the regeneration heat exchanger plates by this arrangement.

The *holding tube* is incorporated into the pasteurizer system to establish the time in this process. The holding tube provides a defined volume in which the continuously flowing milk passes through. When combined with the flow rate of the timing pump, one has a specifically defined, controlled residence time that is part of the pasteurization process. Coupled with the temperature established by the pasteurizer heater section, the time–temperature parameters defined in the pasteurization process are established. To assure this critical control point is met, an indicator thermometer-recorder is located at the outlet of the holding tube along with a flow divert valve. Should the temperature drop below the critical set limit, the flow divert valve will be activated to the "flow diversion" mode and direct the "sub-legal" milk flow back to the balance tank located on the raw side of the system. The commonly used heat treatments and their respective time–temperature parameters for fluid milk will be discussed later in this chapter.

Features of a holding tube will: (1) have a slope up with at least a $\frac{1}{4}$-inch rise per 1 foot of tube length; (2) be free of air drafts; (3) not have attached heat boosters; (4) be under pressure to prevent heat "flashing"; and (5) be assembled in the identical manner prior to its disassembly.

63.4 THE PASTEURIZATION PROCESS (USHHS 1999 Revision; Gösta 2000; Boor and Murphy 2002)

The pasteurization process is simply the heat treatment of raw milk to a specific temperature and held at that temperature for a defined minimal amount of time. The premise for pasteurizing milk is to destroy most disease-producing, nonspore forming bacteria. The targeted microorganism for the process is *Coxiella burnetti*, a pathogen associated with Q-fever. The process is based on a linear, semilogarithmic relationship of temperature to time and its respective lethality on potential disease producing microorganisms.

63.4.1 The Process Parameters

Table 63.2 prescribes the minimal temperature–time relationships as defined by the PMO:

Important to note: The above temperature–time specifications are for milk-based products with a milk fat content of less than 10%. If the milk fat is 10% or more, or if contains added sweeteners, the specific temperature shall be increased by 3°C (5°F).

TABLE 63.2 Temperature–Time Relationships for Milk-Based Products (Min. Fat 10%).

Temperature	Time
Batch/vat 63°C (145°F)	30 min
High temp-short time 72°C (161°F)	15 s
High-temperature 89°C (191°F)	1.0 s
High-temperature 90°C (194°F)	0.5 s
High-temperature 94°C (201°F)	0.1 s
High-temperature 96°C (204°F)	0.05 s
High-temperature 100°C (212°F)	0.01 s

TABLE 63.3 Temperature–Time Relationships for Eggnog.

Temperature	Time
Vat/batch 69°C (155°F)	30 min
80°C (175°F)	25 s
83°C (180°F)	15 s

Provided, that eggnog shall be heated to at least the temperature and time specifications shown in Table 63.3.

Also, *provided further*, that nothing shall be construed as barring any other pasteurization process that has been recognized by the U.S. Food and Drug Administration to be equally efficient and which is approved by the regulatory agency.

63.4.2 Standardizing the Milk Fat Content (Gösta 2000)

As with any business, the market place dictates the types and quantity of the products to be produced. With respect to the fluid milk market place, the highest demand is for skim and low fat milk products. These products have a milk fat content ranging from 0.9% to 2%, respectively. Whole milk is defined having a milk fat level of 3.25%. Thus, when raw milk is received at a milk fat content of between 3.5% and 3.8%, a fat separation process must be employed to remove the fat. Higher milk fat content is usually observed in milk-based frozen-dessert mixes, half and half, and whipping cream.

Standardizing the milk fat content may occur in one of two methods. In multiproduct processing plants where other uses for the milk fat (as cream) are required, the raw milk may be separated at the time of receipt. The skim milk fraction going to a separate storage tank and the cream fraction stored in another. In this case, the cream is blended with the skim milk and homogenized at the standardized fat level for the milk product being processed. In-line sensors are used to monitor the fat content and adjust the blend rate of the cream with the skim milk.

In smaller or narrow focused dairy product manufacturing operations, the stored raw milk passes through a separator after being initially heated to about 60°C (140°F) in the pasteurizer's regeneration section. The separator is adjusted to remove only enough of the cream fraction to meet the fluid milk's product identity. That is, if the raw milk contains 3.6% milk fat and a 2% milk is being processed, the separator is adjusted to remove 1.6% milk fat from that milk stream. The excess milk fat, as thermalized cream, is commonly sold to a butter manufacturing plant.

63.4.3 Minimizing Postpasteurization Contamination

Postpasteurization contamination is a constant challenge to the processing of fluid milk. The most common reason for this contamination problem is a failure in the performance of the prescribed sanitary procedures specifically for pipelines, manually fitted connecting joints, and disassembled parts associated with filling machines. For pipelines, pitting of the metal surface can develop into habitats for biofilm formation. This becomes a growth environment for unwanted bacteria to grow. Manual fitting of connecting joints require gaskets to form a good seal for that connection. Cut gaskets or improper placement of gaskets can lead to a cleaning problem. And with some filling machines requiring disassembly to be cleaned, some machine parts may not receive an adequate

cleaning treatment. As previously discussed with the bacterial problems associated with farm milking equipment, one encounters a similar contamination problem with pasteurized milk. Most often, postpasteurization contamination can be correlated with inadequately cleaned and sanitized equipment and leads to short keeping quality of fluid milk products.

63.4.4 Filling Operations and the Market Milks Produced

Initially, pasteurized fluid milk was placed in returnable glass bottles that required washing at the dairy plant. In the mid-1950s, paper cartons were introduced, followed in the mid-1960s, with the introduction of plastic containers. Today, plastic packaging dominates the filling operations and the paper-board container is the secondary packaging operation. These packaging and filling operations fulfill most of the fluid milk demands that enter commercial channels. However, in the late 1980s, aseptically processed and packaged fluid milk products entered the marketplace with the approval from the USFDA for using hydrogen peroxide as a sterilizing agent for a multilaminated packaging and filling system. In Europe and Asia, aseptic processing and packaging of milk and milk-like products have gained wide acceptance.

63.4.5 High-Temperature-Short-Time (HTST) Pasteurized Fluid Milk Products

Most of the fluid milk products entering major U.S. supermarket chains are HTST processed. Compared to the higher temperature pasteurized milk, the retail shelf-life of HTST processed milk has a shorter keeping quality time and will remain acceptable to the consumer with a possible keeping quality attribute ranging from 5 to 10 days after purchase.

The HTST pasteurization process may affect the shelf-life through its degree of effectiveness in reducing the number of bacteria present in the raw milk's initial microflora. That is, bacteria with thermoduric (heat resistant) properties and spore producing bacteria could survive this pasteurization process. Combine the surviving microflora with a possible post-pasteurization contamination problem and permitting the finished fluid milk's temperature to rise above 4°C (45°F), could lead to a diminished shelf-life product.

63.4.6 Maintaining the Temperature of the Finished Product Below 4°C (40°F)

For all fluid milk products pasteurized under the above stated specifications, maintaining a cold storage condition under 4°C (40°F) prior to and between product use is very important to maintaining its quality attribute. The critical step in the pasteurization process is to bring the finished product temperature to between 2°C (36°F)–3°C (38°F) at the cooling phase of the process. Some product temperature rise can be expected at the filling operation and refrigeration conditions in the dairy plant cooler, during transportation and at the retail store can only maintain the temperature of the product after the process.

63.4.7 In-Container Sterilization

There are countries where cans and glass bottles are prefilled and then retorted to produce a shelf-stable milk. The heat treatments include temperatures from 105°C to 120°C for

20–40 min. Critical to this means of commercially sterilizing milk is the integrity of the container's seal, providing an airtight barrier and controlling the heat process. Predictable heat distribution characteristics of the retort and heat penetration into the product must be known when using this method for sterilizing fluid milk.

63.5 ASEPTIC PROCESSING AND PACKAGING OF FLUID MILK (EXTENDED SHELF-LIFE MILK) (Chambers and Nelson 1993; Gavin and Weddig 1995; Boor and Murphy 2002)

63.5.1 The Concept

The initial concept for aseptic processing has been credited to C. Olin Ball, a pioneer in developing the thermal processing concepts used worldwide today. Ball referred to the aseptic process as the heat-cool-fill (HCF) process. The first commercial HFC process used was in California where metal containers were filled with a cream product, then sterilized in a retort followed by a seaming operation where a steam sterilized metal lid was applied. This process was known as the Avoset process. The Dole aseptic process followed the Avoset system and provided the prototype, continuous process scheme now used by the food industry. Until FDA's approval of hydrogen peroxide as a chemical sterilant for packaging material, steam was the primary method used for sterilizing the food package, a metal container. This sterilized container was brought into a sterile filling environment, filled with the sterile product and then sealed with a steam sterilized lid.

63.5.2 Aseptic Processing and Packaging: The System

The concept for aseptic processing and packaging is simple. Sterilize the milk/food product and package separately, then place the milk/food material into the package under aseptic conditions and seal the package. Bridging the gap between concept and practice is the challenge. From the time the raw food materials are received, have passed through the process equipment and are placed into the package and sealed, many critical performance factors must be under control within the processing environment. At each phase of milk handling, processing and packaging, all aspects of the Good Manufacturing Practices and Hazardous Analysis Critical Control Points plan should be fully implemented to assure the safety and quality of the fluid milk products processed. Implementation should emphasize four essential sectors of the processing environment. These sectors are: (1) equipment design and operation; (2) written operating procedures which include good manufacturing practices, material handling protocols, instructions for the proper operation of handling and processing equipment, product formulations, and quality assurance critical control points; (3) proper training of operations personnel; and (4) regulatory compliance.

63.5.3 Equipment Design and Operation

Critical to the aseptic system's equipment design is its ability to hold a positive pressure from the positive displacement pump at the beginning of the system through to the back pressure valve just prior to the product surge tank. After the back pressure valve or pump, a slight pressure (usually 1–3 psig) is maintained on the product surge tank and pipeline to assure unsterile air can not be drawn into the sterile product as it proceeds on to the filling operation. Figure 63.1 presents a typical aseptic processing scheme. This scheme identifies

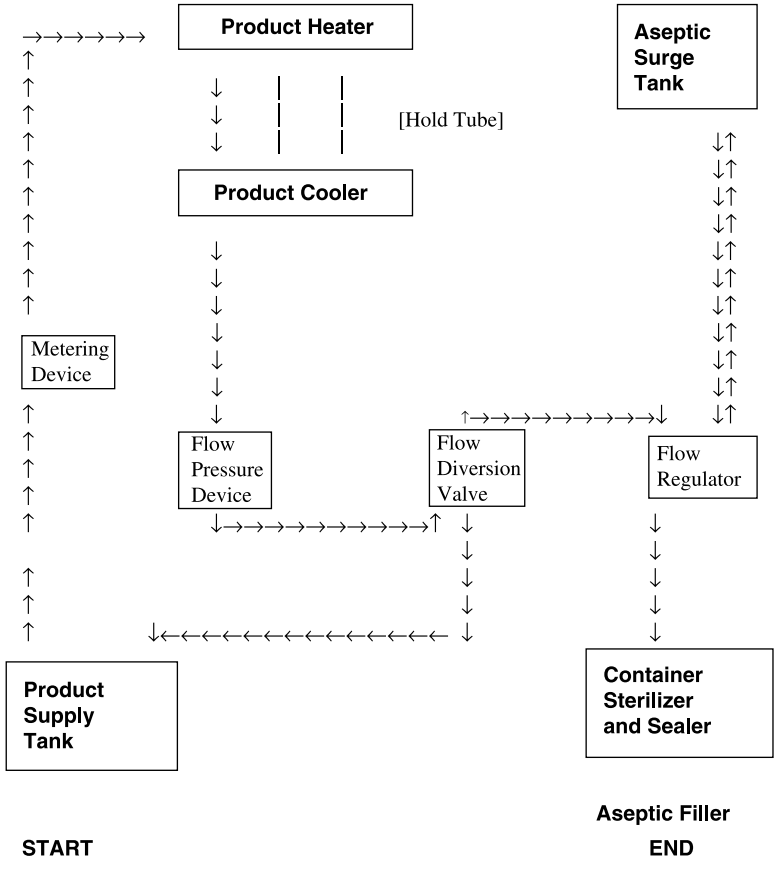

Figure 63.1 *Aseptic processing: a simplified scheme; ©2006, Science Technology System, West Sacramento, CA. Used with permission.*

several important unit operations that must function properly to process a safe and commercially sterile food product. As this aseptic system is assembled, three important design features must be incorporated into the construction of the system. The first design feature is to provide protective barriers between the internal, sterile sections of the equipment and the operating environment. Two critical unit operations where this feature is applied are the positive displacement pumps using steam tracers and a condensate bleeder and the valve assemblies where valve stems reciprocate into the sterile environment and may use sterile condensate, sterile air, or iodine or chlorine as the protective barrier. The second design feature that must be engineered into the aseptic operation is a gas/air filter system or a hyperfilter. Filter systems are placed down stream from the back-pressure valve and at the surge tank or bulk storage tank, depending on the application. Filter systems should require at least two filtering units placed in series at the gas/air inlet of the application site. The third design feature must assure the sealing integrity of connecting or joint applications. Two considerations must be given to assuring this sealing integrity. These are selection of the proper gasketing material as determined by the specific application and the method of connecting unit operations and pipelines (e.g., welding,

clamp joints). Dairy-style threaded joints are not recommended for use in aseptic processing applications.

The next important consideration for the aseptic operation is the thermal process. This operation is a critical control point in the system and must be designed carefully. There are two basic means for transferring heat into a milk-based product, namely, direct and indirect. If a direct heating method is used where steam is injected into the milk then either the dairy product formulation must compensate for the added water or a dewatering/flash unit operation must be used. For indirect heating, it is recommended that the milk undergo a deaeration step prior to entering the heat exchanger to remove undesireable air. The heat exchanger selection is determined by the viscosity characteristics of dairy product heated. For example, liquids that have Newtonian flow characteristics can be heated effectively through plate and tubular type heat exchangers. In contrast, higher viscous, homogenous materials such as ice cream and soft-serve mixes might require a scrape surface heat exchanger design for heating purposes.

Ultra-high-temperature (UHT) processing is commonly associated with aseptic processing. This is because the temperature range (130–150°C) utilized targets its lethality to both non-spore forming and spore forming bacteria that leads to a commercial sterilization of the dairy product. Holding times inversely decrease (vary from 8 s to 2 s) as the temperature increases to assure a nine-log reduction of thermophilic endospores and to ensure a 12-log reduction for *Clostridium botulinum*. UHT pasteurized/sterilized dairy products are aseptically packaged and then held under refrigeration during distribution into commercial retail channels. Usually, a 10-week shelf-life can be expected for UHT pasteurized milk often referred to as "extended-shelf-life milk."

In contrast, UHT sterilized milk will use temperature treatments above 135°C with a hold period of at least 2 s to assure adequate lethality. The sterilized milk is aseptically packaged but is distributed and sold under nonrefrigerated conditions.

Complimenting the heating method selected are four components required to monitor the thermal process. These components are: (1) the heat monitoring and controlling devices; (2) the heated milk's retention method; (3) a means to divert thermally underprocessed product back to the front of the process system (required for dairy products); and, (4) monitoring the pressure differential across the regeneration section of the heat exchanger. Since all thermal processes involve a temperature and time relationship, these operating parameters must be verified and constantly monitored to assure the established process has been achieved. In addition, most public health agencies that enforce regulations governing the thermal processing of low acid and acidified foods require records be kept and maintained on this critical control point. Typically, four components are engineered into the thermal process to assist in monitoring and documenting the temperature and time parameters. Essential to the process is the placement of the indicating temperature measuring device which must be referenced to the official temperature measure instrument, the mercury-in-glass thermometer. This temperature-indicating device must be placed at the end of the holding tube or bottom of the flow equalization/holding tank where the minimum hold time is determined. A thermometer or temperature indicating device is also required at the inlet of the detention unit to monitor temperature fluctuation of the heat exchanger. Included in the regulations is that a temperature recording and controlling device be installed to monitor and record the time–temperature parameters, and to initiate control of the heating medium if the temperature approaches a low or high critical limit. This same controlling device also activates the flow diversion valve at the end of the time detention unit if the temperature decreases below a predetermined low critical value. The

recording chart paper must be appropriate for the recording device used and standardized against the reference temperature-measuring instrument (the indicating thermometer) and an accurate timing device (analog clock with a sweep second hand). Positive displacement pumps are always used as the timing pump for aseptic processing. If this pump is a variable speed pump, the speed control of the pump, once set at a critical pumping rate, must be sealed against unauthorized adjustments to higher speeds. The fourth component of the thermal process is to provide a positive pressure (at least 1 psig) differential on the sterile product side of a plate where the heat exchanger utilizes a heat regeneration section.

63.5.4 Packaging Platforms

With approval of hydrogen peroxide as a packaging sterilant and its use in combination with heat or ultraviolet light to produce a reliable method for sterilizing packaging materials, several packaging systems have been introduced commercially to the dairy and food processing industry.

1. Thermoform-fill and seal
2. Form-fill plastic pouches
3. Web-fed paperboard laminates and plastic containers
4. Preformed bags
5. Blow-molded containers
6. Partially formed laminated paper containers
7. Preformed rigid and semirigid containers such as:
   a. Glass
   b. Metal
   c. Drums
   d. Composite
   e. Plastic cups and bottles

Advantages gained through the use of aseptic processing and packaging are that the milk, beverages, and food products processed, using this technology, deliver improved flavor, taste, or eating quality when compared to conventional thermally processed foods. The reason for this improved quality is the rapid movement of heat into and out of the product during the thermal process which results in a minimum chemical change to the food materials. Aseptic packaging add some additional benefits such as: is a less expensive packaging form; light weight materials can be used; is environmentally sound; and, can be easily transported and handled with a reduction in package damage.

63.5.5 Milk System Stability Considerations and Product Shelf Life (Richardson 1983; Kohlman and others 1988; Gösta 2000)

There are shelf life quality attributes and milk system stability considerations associated with aseptically processed milk. The major consideration is the “gelation phenomenon” experienced with aseptically processed milk. Research by Richardson (1983) and Kohlman and others (1988) have defined the probable milk system and mechanism involved with this phenomenon. It appears that the milk’s plasmin system consisting of native proteases and associated activators and inhibitors are responsible. The

denaturation of the plasminogen activators and the plasmin inhibitor by the heat process allows the milk plasminogen to convert to plasmin, thus, permiting the native, active proteases to breakdown the milk's casein. The result, a product shelf-life of 6–9 months due to gelation of the milk in the package. Other milk quality attributes affected are cream-lining, increased viscosity, sediment, Maillard reactions related to browning, and flavor changes.

63.6 WRITTEN STANDARD OPERATING PROCEDURES

Written standard operating procedures must include good manufacturing practices, material handling protocols, product formulations, instructions for the proper operation of handling and processing equipment, and enforcement of the hazardous critical control points as well as product quality control functions.

Special good manufacturing practice considerations for aseptic processing and packaging operations should include: a commitment to a "clean room" processing environment; close inspection of the food contact surface areas for cleanliness; and an effective sanitation program which includes steam sterilizing the aseptic processing lines and equipment. An effective GMP program contributes to the protection against and prevention from environmental contaminants gaining entry into the food materials. Considerable effort goes into establishing a sterile environment within the equipment, pipelines, and packaging operations that is further validated by bacterial spore challenge tests. Where food soil is permitted to build up on the heat transfer surface areas, fouling will develop that reduces the overall heat transfer efficiencies of the heat exchanger. Other problems attributed to fouling are flavor changes, system pressure build-up and loss of energy transfer.

How raw milk, its ingredients, labels, packaging materials, and other support materials are received, examined, accepted, and stored for use in the processing environment can have a very significant influence on the performance of the processing line and the final product quality. Materials that do not meet established performance specifications and slip through the critical receiving step or are improperly handled and stored can lead subsequential production problems. For example: storage conditions that subject food materials to excessive heat and moisture can accelerate deterioration of the food quality through oxidative and enzymatic mediated chemical changes and microbial growth; not meeting critical microbial specifications can result in high heat resistant spore populations being present in ingredients such as starches and sugars which can introduce spoilage organisms into the food product which survive the thermal process; and, careless labeling of opened ingredient bags can lead to possible cross mixing with other chemicals used for nonfood applications that could result in individuals being injured.

63.6.1 Employee Training

The productivity and efficiencies of any production system greatly depend on the people who operate the production lines, formulate and blend the ingredients of the finished product, operate the processing equipment, fill the food package, and provide the clean and sterile equipment. When everyone does their job correctly, the end result is a safe, high quality food product. However, everyone must know what is expected of them and they need to be properly trained in the execution of their assigned tasks. The main

point of reference for these tasks should be written procedures that clearly explain the purposes of each task and operation and who is responsible for each of those functions. As GMPs and Hazardous Analysis Critical Control Points are incorporated into those written procedures, the following criteria should be defined: (1) the hazard at issue should be identified (i.e., biological, chemical, or physical); (2) critical control points (CCP) must be identified and the controlling mechanism, process or agent (i.e., ultraviolet light) specified; (3) state what parameter(s) is/are to be monitored and recorded with critical value limits established so that proper interventions can be initiated; and, (4) identify who is/are responsible for correcting the out-of-control CCP and the follow up required to be sure the CCP is under control.

In the aseptic processing and packaging of food materials, the obvious hazard that the majority of the CCPs are targeted to is the biological/microbial hazard. Thus, on the thermal processing side of the operation, the production personnel would monitor for: (1) the temperature of the heated product at selected sites (as previously discussed); (2) a constant pressure level from the heat exchanger to the back pressure valve and the positive pressure between that valve and the filling unit, including the surge tank; (3) the timing pump speed; (4) the time–temperature profile as indicated on the chart recording; (5) air filter flow rates and pressures for the surge tank; and, (6) equipment malfunctions. Another important function of the thermal process equipment operator is to standardize the temperature of the recording device and to check the performance of the flow diversion valve at the critical low temperature limit.

For the packaging side of the aseptic system, the line production personnel would monitor: (1) the aseptic zone, established between the heating section of the heat source and the package sealing environment, for violation of that zone through operator error, malfunctioning process equipment, or filler/package failure; and (2) the package sterilization process which includes heat, hydrogen peroxide and irradiation as sterilant agents. During the package sterilizing step, it would be important to monitor the consumption rate of the hydrogen peroxide, its concentration, package contact time, and temperature. Package sealing integrity testing and measuring for hydrogen peroxide residuals are CCPs for aseptic packaging since these monitor for a biological and chemical hazard, respectively.

63.6.2 Compliance with Public Health Regulations (USHHS 1999 Revision)

The implementation of GMPs and HACCP in the dairy processing environment improves the reliability of that environment to manufacture a safe, wholesome commercial food product. These sanitary practices, monitoring and controlling activities help to protect and prevent accidental contamination or underprocessing of food products that otherwise might reach commercial channels. Thus, the well being of the consumer is protected from eating unsafe food products as the requirements of these principles are put into practice. Because of the public health implications of this important benefit, these principles and practices have been and are being incorporated into many of the food manufacturing regulations by most of the industrialized nations around the world. U.S. regulations governing the use of the aseptic processing and packaging technologies can be found in the Code of Federal Regulations under Title 9 and 21. These Title numbers are assigned to all regulations enforced by the U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA), respectively. The USDA regulations covering aseptic

processing and packaging can be found under 9 CFR 318.3 and 9 CFR 381.3. USDA must approve the use of aseptic related equipment and procedures used in meat and poultry processing operations. In contrast, FDA only accepts or rejects process filling forms but does not approve the equipment used in aseptic processing and packaging operations. The FDA regulations for the thermal processing of low acid foods (21 CFR 113) and acidified foods (21 CFR 114) and the GMPs requirements (21 CFR 110) are also applicable to the aseptic processing and packaging technologies used in food processing applications.

63.7 A FINAL COMMENT

The information provided in this chapter discusses the minimal operating standards for a fluid milk collection, handling, and processing environment. Local handling and storage conditions may require additional strategies to protect the product quality and safety. That is the responsibility of the dairy company producing the products. It is imperative that production, operational, and management personnel adhere to the principles conveyed in the references provided below and compliance with the U.S. Code of Federal Regulations and the respective State's milk ordinances and regulations to assure a safe milk-based product.

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64

Milk Composition, Physical and Processing Characteristics

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64.1 Introduction 348
64.2 Definition of Milk 349
64.3 Milk Composition 349
64.3.1 Factors Affecting Composition of Milk 349
64.4 Physical Structure 350
64.5 Constituents of Milk 351
64.5.1 Major Constituents 351
64.5.1.1 Water 351
64.5.1.2 Fat 351
64.5.1.3 Proteins 355
64.5.1.4 Lactose 362
64.5.1.5 Minerals 363
64.5.2 Vitamins and Some Other Minor Constituents 365
64.6 Physical Characteristics of Milk 366
64.6.1 Optical Properties 366
64.6.1.1 Color 366
64.6.1.2 Refractivity 367
64.6.2 Flavor 367

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64.6.3 Acidity and pH 368
64.6.4 Buffering Capacity 369
64.6.5 Electricochemical Properties 370
64.6.5.1 Oxidation Reduction Potential 370
64.6.5.2 Electrical Conductivity 370
64.6.6 Thermal Properties 370
64.6.6.1 Thermal Expansion 370
64.6.6.2 Heat Capacity 370
64.6.6.3 Specific Heat 371
64.6.6.4 Thermal Conductivity 371
64.6.6.5 Effects of Heat 371
64.6.6.6 Heat Stability 372
64.6.7 Density and Specific Gravity 373
64.6.7.1 The Density 373
64.6.7.2 The Specific Gravity 373
64.6.8 Viscosity 374
64.6.9 Surface Activity 374
64.6.9.1 Foaming 375
64.6.10 Curd Tension 375
64.6.11 Colligative Properties 376
64.6.11.1 Osmotic Pressure 376
64.6.11.2 Freezing Point 376
64.6.11.3 Boiling Point 377
References and Further Reading 377

64.1 INTRODUCTION

From physiological standpoint, milk is a unique biological secretion of the mammary gland endowed by nature to fulfill the entire nutritional needs of the neonate. Following parturition, milk is the secretion of normally functioning mammary gland of the females of all mammals. The yield and composition of milk varies among various species to entirely meet postnatal growth requirements of the offspring. Milk, therefore, contains all the chemicals in the form of six major nutrients, namely, water, fat, proteins, carbohydrates, minerals, and vitamins that are ideal for nourishment. Milk and milk products are used as components of many food products around the world.

Milk is an integral part of fermented milks including yogurt and considered by many as an ideal vehicle to deliver beneficial cultures as well as probiotics and ingredients known to stimulate activity of these organisms and the microflora of human gastrointestinal tract. The conversion of milk into fermented milks augments the nutritional value of inherent milk constituents. Additionally, fermentation process generates metabolic and cellular compounds which have positive physiological benefits for the consumer.

This chapter provides basic information relative to milk composition that is relevant to the processing of milk and dairy products. For detailed discussion, the reader is referred to Wong and others (1988); Jensen (1995); Swaisgood (1996); Fox and Sweeney (1998); and Walstra and others (1999).

64.2 DEFINITION OF MILK

Chemically speaking, milk is a complex fluid in which more than 100,000 separate molecules and chemical entities have been found, the levels of which vary with the species. In terms of physical chemistry, milk is an opaque, white heterogeneous fluid in which various constituents are held in multidispersed phases of emulsion, colloidal suspension, or solution.

Worldwide, milk from cows, water buffaloes, goats, sheep, camels, mares, and other mammals is used for human consumption. However, cows' milk entails by far the most important commercial significance.

According to the Food and Drug Administration of the United States, milk refers to cows' milk. Milk from other species must be labeled to indicate the species. For instance, milk from goats must be called goats' milk.

Milk is the whole, clean lacteal secretion of one or more healthy cows properly fed and kept, excluding that obtained within 15 days before calving and 3–5 days after. This would exclude colostrum, the milk secreted immediately after giving birth. The definition of Grade A milk as per FDA standards of identity is "the lacteal secretion practically free of colostrum, obtained by complete milking of one or more healthy cows, which contains not less than 8.25% milk solids – not fat to solids-not-fat less than 3.25% milk fat."

64.3 MILK COMPOSITION

Chemical make up of milk and its physicochemical behavior provide scientific basis for basic processing of milk and manufacture of products. The composition of milk is generally described in terms of its commercially important constituents, milk fat, and nonfat solids (NFS) or milk solids-not-fat (MSNF). The MSNF consists of protein, lactose and minerals. These solids are also referred to as "serum solids." The term "total solids" refers to the serum solids plus the milk fat. The major constituents of milk are given in Table 64.1.

The ash content is not quite equivalent to salt level in milk. In the determination of mineral content, some salts like chlorides and organic salts are volatilized or destroyed as a result of high-temperature exposure during routine mineral analysis by the ash method. The data given in Table 64.1 refers to all major breeds of cows in North America. Milk from Jersey and Guernsey breeds would be closer to higher fat and protein range.

64.3.1 Factors Affecting Composition of Milk

Apart from the differences due to the breed, certain additional factors also influence the gross composition of milk: (1) individuality of animal; (2) stages of milking; (3) intervals of milking; (4) completeness of milking; (5) frequency of milking; (6) irregularity of

TABLE 64.1 Composition of Bovine Milk.

	Water	Fat	Protein	Lactose	Ash
Average, %	86.6	4.1	3.6	5.0	0.7
Range, average %	84.5–87.7	3.4–5.1	3.3–3.9	4.9–5.0	0.68–0.74

Source: Swaisgood (1996).

milking; (7) portion of milking; (8) different quarters of udder; (9) lactation period; (10) yield of milk; (11) season; (12) feed; (13) nutritional level; (14) environmental temperature; (15) health status; (16) age; (17) weather; (18) oestrum or heat; (19) gestation period; (20) exercise; (21) excitement; and (22) administration of drugs and hormones. In general, these variables tend to average out in commercial pooled milk used by dairy processors, but it does display an interesting seasonal pattern. The seasonal variations in protein and mineral content have an important impact on viscosity and gel structure of yogurt and fermented products. During late spring and early summer months, milk in some areas of the United States registers low protein and calcium content resulting in poor viscosity in finished yogurt. During these months of low protein milk, it is necessary to compensate by raising the solids-not-fat content of yogurt mix by 0.25–0.50%.

64.4 PHYSICAL STRUCTURE

Various interactive forces between the chemical constituents of milk determine the technological behavior of milk. Milk has well-defined physical equilibrium between various constituents that exist mainly in three forms, namely, emulsion, and colloidal and true solution. Milk lipids are present as an "oil-in-water type," of emulsion in the form of microscopic globules varying from 0.1 to 22 μm in diameter. The colloidal phase contains casein micelles, calcium phosphates, and globular proteins. Whey proteins are in colloidal solution and the casein is in colloidal suspension. Lactose, vitamins, acids, enzymes, and some inorganic salts are present as true solutions. Table 64.2 gives the relative sizes of these particles in milk.

Certain factors tend to influence the physical equilibrium of milk that exists between colloidal dispersion and salts. These factors are: (1) addition of polyvalent ionizable salts; (2) concentration of serum solids; (3) changes in pH; (4) heat treatment (which may alter the surface charges or unfold proteins); (5) addition of alcohols (which reduces bound water associated with the colloidal constituents). All these factors tend to destabilize colloidal systems and thus influence the technological behavior of milk during product manufacture. In the production of cultured milks, as the fermentation proceeds, the colloidal calcium phosphate gets progressively converted to ionic form as the pH drops from 6.6 in milk to less than 4.6 in yogurt and fermented milks. Casein and the interacted whey proteins coagulate at the isoelectric point at pH 4.6, forming gelled structure.

Certain terms related to milk structure need clear understanding. Milk plasma is the fluid portion of milk minus fat globules being almost similar to skim milk. Milk serum is milk plasma minus milk fat and casein micelles. Removal of casein micelles

TABLE 64.2 Physical State and Particle Size Distribution in Milk.

Compartment	Size, Diameter (nm)	Type of Particles
Emulsion	2000–6000	Fat globules
Colloidal	50–300	Casein-calcium phosphate
Dispersion	4–6	Whey proteins
True solution	0.5	Lactose, salts, and other substances

from skim milk by clotting with rennet yields the liquid called whey. It is different from milk serum because it contains some polypeptides cleaved from casein by rennet.

64.5 CONSTITUENTS OF MILK

64.5.1 Major Constituents

64.5.1.1 Water. Water is the medium in which all the other components of milk (total solids) are dissolved or suspended. Water content varies from 85.4% to 87.7% in different species of cows (Table 64.1). A small percentage of the water in milk is hydrated to the lactose and salts and some is bound with the proteins.

64.5.1.2 Fat. Milk fat though, quite bland in taste, imparts richness/smoothness to fat containing dairy products. Milk fat in freshly secreted milk occurs as microscopic globular emulsion of liquid fat in aqueous phase of milk plasma. Fat content of milk varies from 3.4% to 5.1%, depending on the breed of the cow. Most of the raw milk used for dairy processing typically contains an average of 3.5–3.6% fat. Variability of milk fat also depends upon the individuality of animal, stage of lactation, feed, environmental factors, and stage of milking. The composition of milk fat is given in Table 64.3.

The milk fat of cows consists chiefly of triglycerides of fatty acids, which make up 95–96% of milk fat. The remaining milk fat is composed of percent diglycerides, monoglycerides, free fatty acids, phospholipids, and cholesterol as shown in Table 64.3.

The functional properties of milk fat are attributed to its fatty acid make up. More than 400 distinct fatty acids have been detected in milk. Typical milk fat consists of 62% saturated, 29% monounsaturated and 4% polyunsaturated fatty acids. It contains 7–8% short chain fatty acids (C_4–C_8), which is a unique characteristic of milk fat. The major fatty acids of milk fat are given in Table 64.4.

Milk fat functions as a concentrated source of energy as well as a source of fat-soluble vitamins A, D, E, and K and essential fatty acids, linoleic, and arachidonic acids. The essential fatty acids are not synthesized by human body. They must be supplied by the diet. Arachidonic acid with four double bonds is present in traces. Its precursor is linoleic acid. Omega-3-linoleic acid and its products EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are also present in trace but significant amounts. The positional location of individual fatty acids in the triglycerides is not random. In fact, the *syn*-1 and *syn*-2 positions on the glycerol molecule are mainly occupied by myristic ($C_{14:0}$),

TABLE 64.3 Composition of Bovine Milk Fat/Lipids.

Lipid Fraction	g/L in Milk	Weight %
Triacylglycerols/triglycerides	30.7	95.80
Diacylglycerols/diglycerides	0.72	2.30
Monoacylglycerols/monglycerides	0.03	0.08
Free fatty acids	0.09	0.28
Phospholipids	0.36	1.11
Cholesterol	0.15	0.46
Cholesterol esters	0.006	0.02
Total	32.056	100.05

TABLE 64.4 Fatty Acid Profile of Milk Fat.

Fatty Acids	Common Name	Weight
$C_{4:0}$	Butryic	3.8
$C_{6:0}$	Caproic	2.4
$C_{8:0}$	Caprylic	1.4
$C_{10:0}$	Capric	3.5
$C_{12:0}$	Lauric	4.6
$C_{14:0}$	Myristic	12.8
$C_{14:1}$	Myristoleic	1.6
$C_{15:0}$	–	1.1
$C_{16:0\ (branched)}$	–	0.30
$C_{16:0}$	Palmitic	43.7
$C_{16:1}$	Palmitoleic	2.6
$C_{17:0}$	–	0.34
$C_{18:0\ (branched)}$	–	0.35
$C_{18:0}$	Stearic	11.3
$C_{18:1}$	Oleic	27.42
$C_{18:2}$	Linoleic	1.5
$C_{18:3}$	Linolenic	0.59

palmitic ($C_{16:0}$), stearic ($C_{18:0}$), or oleic acids ($C_{18:1}$). The *syn*-3 positions contain butanoic ($C_{4:0}$), hexanoic ($C_{6:0}$) or oleic ($C_{18:1}$) acids.

Saturated fatty acids are solid at ambient temperature, while unsaturated fatty acids are liquid. Their ratio in milk fat has a profound effect on the hardness and spreadability of butter at low temperatures. The balance between C_4 and C_{18} fatty acids keeps milk fat liquid at body temperature (Otter 2003). The origin of fatty acids is either blood plasma lipids or they are synthesized in the mammary gland. There is a correlation between the fatty acid composition of feed lipids and butter hardness. A seasonal effect is seen as well. A softer butter is observed when the cow is on summer pasture or when the ration includes oils liquid at ambient temperature.

Cholesterol. The cholesterol content of milk is significantly affected by the species, breed, and feed, stage of lactation, and season of the year. Cholesterol content is generally lowest in the beginning of lactation period and progressively rises throughout the lactation period being highest towards the end of the lactation. The cholesterol content of colostrum is relatively high (570–1950 mg per 100 g fat) for the first milking after parturition and progressively declines to normal levels during subsequent milking.

In general, typical cholesterol content of whole milk (3.25% fat) is 10.4 mg/100 mL or 24.4 mg per serving of 8 fl. oz. It corresponds to 3–4 mg/g fat. Fat reduction in dairy products is accompanied by cholesterol reduction. By separating fat from milk, an 80% reduction in cholesterol content can be achieved in skim milk. Thus, nonfat milk/skim milk shows residual cholesterol level of 4.9 mg/8 oz serving. Dairy products, therefore, contain cholesterol content depending on the milk fat and nonfat solids content of the product.

Phospholipids. A number of factors influence the unique phospholipid content of milk. The total phospholipid content of cow's milk is approximately 36 mg/100 mL.

Milk Fat Globule. Milk fat occurs in milk as an emulsion of fat particles suspended in aqueous phase. The spherical particles are called fat globules (Fig. 64.1).

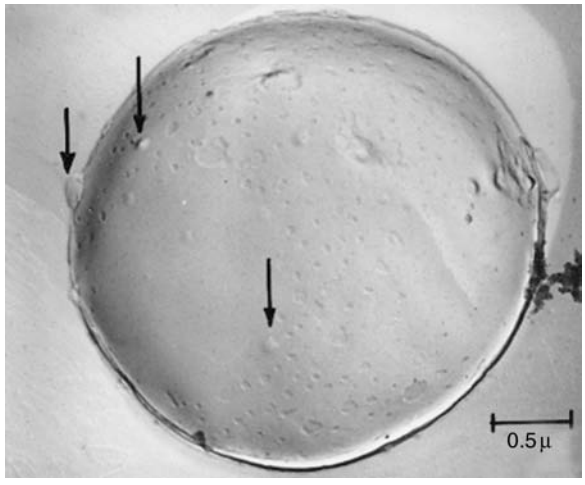

Figure 64.1 *Electron micrograph of freeze-etched fat globule of Jersey cow milk. Note the surface is smooth as well uneven, and some particle-like material protrudes out on the surface as depicted by arrows.*

The average size of fat globules in raw cow milk varies from 3.4 to 4.5 μm, depending on the breed of the cow. Jersey milk tends to have larger diameter than Holstein milk. Milk lipid globules fall into three overlapping size distributions (Table 64.5).

The use of a separator in dairy plants permits fractionation of whole milk into skim/low fat milk and cream. Fat globules are lighter (less dense) than the surrounding water phase and rise to the surface when milk is left undisturbed as per Stoke's law.

$$V = \frac{2r^2(\text{density of serum} - \text{density of fat}) \times g}{\text{Viscosity of milk} \times 9}$$

where, V is velocity of rise of fat globules, g is the gravitational force and r is the diameter of the fat globule. From the equation, it follows that V is directly proportional to g. If g is increased by centrifugal force, fat globules can be separated in a relatively short time. Also, g is inversely proportional to the viscosity of milk which decreases as the temperature goes up converting the fat into liquid state. Accordingly, V is increased. Thus, separation is more efficient at warmer temperatures. Skim milk should contain 0.05% fat or less, if the separator is functioning properly.

Processed milk products, namely homogenized milk, UHT milk, ice cream, yogurt, light cream, half and half, evaporated milk, and condensed milk, which have undergone homogenization have diameter of their globules of the order of 0.3–0.7 μm. In the

TABLE 64.5 Size Distribution of Milk Lipid Globules.

Class	Diameter (μm)	Proportion of the Total Globule Population (%)	Fraction of Total Milk Lipid (%)
Small	Below 2	70–90	<5
Intermediate	3–5	10–30	90
Large	8–10	0.01	1–4

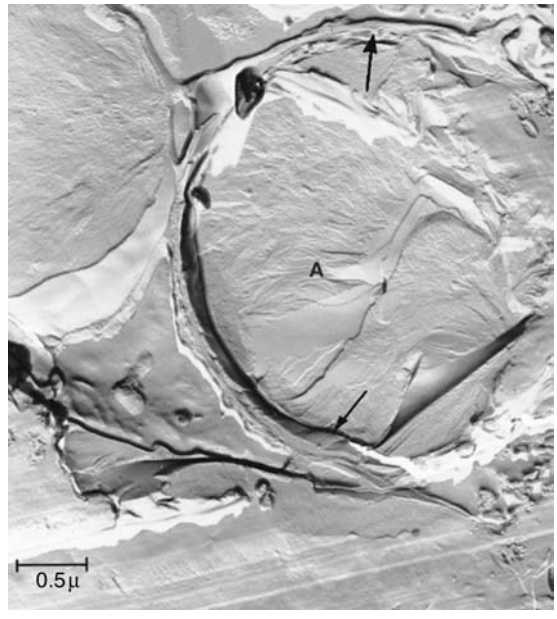

Figure 64.2 *Electron micrograph of freeze-etched fat globule membrane isolated from the globules of Jersey cow milk. Note the membrane as depicted by arrows.*

unhomogenized products like whipping cream fat globules show a diameter of 4.0 μm. Skim milk has smaller fat globules left over as a result of separator action and their diameter is around 1.3 μm. Cream layer is observed in products with relatively large fat globules, while the homogenized dairy products show virtually no cream layer during the shelf life of such products.

The fat globules are stabilized by a very thin membrane, closely resembling plasma membrane, only 5–10 nm thick (Fig. 64.2).

The fat globule membrane consists of proteins, lipids, lipoproteins, phospholipids, cerebrosides, nucleic acids, enzymes, trace elements, and bound water, details of which are given in Table 64.6.

The membrane is important in keeping the fat from separating as free oil when it is subjected to physical abrasion during handling/processing of milk. It also protects milk lipids against the action of enzymes, notably lipase, in development of rancidity. Certain

TABLE 64.6 Proximate Composition of Bovine Milk Fat Globule Membrane.

Component	%(w/w) of Total Membrane
Protein	41
Phospholipids	27
Neutral glycerides	14
Water	13
Cerebrosides	3
Cholesterol	2

Source: Fox and McSweeney (1998).

enzymes, such as the alkaline phosphatase and xanthine oxidase as well as certain important minerals as iron and copper are preferentially attached to the fat globule membrane. The membrane contains 5–25% of the total copper and 30–60% of total iron content of milk. Other elements associated with membrane are cobalt, calcium, sodium, potassium, magnesium, manganese, molybdenum, and zinc. Molybdenum is associated with the enzyme xanthine oxidase. Activity of nearly all the enzymes of milk has been detected in the membrane.

The proteins of membrane are unique and are not found in skim milk phase. Due to damage of the globule or as a result of homogenization, the membrane proteins contain skim milk proteins (casein and whey proteins). A hydrophobic protein, butyrophilin, has been isolated from the membrane, which shows extraordinary affinity for association with lipids.

The lipid fraction of the membrane constitutes about 1% of the total milk lipids. It contains phospholipids and neutral lipids in the ratio of 2 : 1. The phospholipids are phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin in the ratio of 2 : 2 : 1 (Fox and McSweeney 1998). The major fatty acid content of phospholipids is 5% $C_{14:0}$, 25% $C_{16:0}$, 14% $C_{18:0}$, 25% $C_{18:1}$, 9% $C_{18:2}$, 3% $C_{22:0}$, and 3% $C_{24:0}$. Accordingly, unsaturated content of the membrane lipids is different from rest of milk lipids in terms of their high unsaturated fatty acid level. Thus, they are more susceptible to oxidative deterioration.

The neutral lipids of the membrane consist of approximately 83–88% triglycerides, 5–14% diglycerides, and 1–5% free fatty acids. The fatty acids contained therein are largely long chain. In order of their preponderence, they are palmitic, stearic, myristic, oleic, and lauric acids.

The sterols, vitamin A, carotenoids, and squalene are largely located in the fat core of the globule.

64.5.1.3 Proteins. Milk contains hundreds of proteins and most of them occur in trace amounts. The major proteins of milk are broadly classified as caseins and whey proteins. Caseins are defined as the proteins which are insolubilized and precipitate when the pH of milk is lowered to 4.6. The soluble fraction at pH 4.6 is termed as whey proteins. In addition, milk contains degradation products by plasmin, an inherent proteolytic enzyme. Thus, γ-casein and proteose peptones owe their origin to proteolysis of β-casein. Also, proteins derived of milk fat globule membrane are present. The membrane proteins are spilled into milk system following mechanical disruption of the fat globule, such as churning and homogenization processing. Milk also contains numerous enzymes, and biologically active proteins. Nonprotein nitrogen compounds like urea, uric acid, creatine, creatinine, orotic acid, and hippuric acid are also found.

Casein, the principal milk protein, makes up 80% of the total while whey proteins make up the remaining 20%. These fractions have been shown to be heterogeneous consisting of several proteins (Table 64.7).

Caseins. Typical of milk proteins, caseins display distinctive structure, charge, physical, and biological properties as well as nutritional role. The interaction of various caseins and calcium phosphate contributes to the formation of large colloidal complex particles called casein micelles. The whitish color of milk is ascribed to light scattering effect of colloidal micelles. The micelles are rough surfaced spherical particles varying in size from 50 to 500 nm. Electron picture analysis has shown that the micelles are composed of smaller particle or submicelles of 20 nm diameter or less. Hydrophobic interactions with

TABLE 64.7 Concentration of Various Proteins and Polypeptides in Milk.

Protein/Polypeptide	Concentration in Milk (g/100 mL)
Caseins	2.4–2.8
α_{S1}-Casein	1.2–1.5
α_{S2}-Casein	0.3–0.4
β-Casein	0.9–1.1
κ-Casein	0.3–0.4
Casein fragments	0.2–0.35
γ-casein	0.1–0.2
Whey proteins	0.5–0.7
β-Lactoglobulins	0.2–0.4
α-Lactalbumins	0.1–0.17
Serum albumins	0.02–0.04
Immunoglobulins	0.05–0.18
Proteose peptone	0.06–0.17
Milk fat globule membrane protein	0.04
Enzymes	–

calcium phosphate and submicelles seem to be involved in the formation of micelles. Micelle composition consists of 63% moisture and the dry matter consists of 92–94% protein and 6–8% colloidal calcium phosphate. Other associated salts are magnesium and citrate. Micelles have a porous structure with large voluminosity (approximately 4 mL/g of casein. They are considerably hydrated, showing 3.7 g water/g casein.

Caseins are further divided into α_{s1}-, α_{s2},-, β-, and κ-fractions (Table 64.7), which along with whey proteins, β-lactglobulin and α-lactalbumin, are gene derived proteins synthesized in the mammary gland. All these proteins are heterogeneous and exhibit genetic polymorphs. There are two to eight genetic variants differing from each other in one to 14 amino acids. The variants may have impact on the protein concentration and processing properties of milk. The γ-fraction is derived from the break down of β-casein by the native proteolytic enzymes of milk.

The caseins are phosphorylated proteins, containg one to 13 phosphoserine residues. κ-Casein exists in as many as nine glycosylated forms. It contains two cysteine molecules per molecule. As a result of disulfide bond formation, it can exist as polymers of two to eight units. Similarly, α_{S-2}-casein also contains two cysteines and exists in a dimeric form. The composition and size of various caseins are shown in Table 64.8.

Casein micelles contain α_{s1}-, α_{S2}-, β-, and κ-casein in the ratio of 3 : 1 : 3 : 1. Most of the fractions α_{s1}-, α_{S2}-, and β-casein are located in the interior of micelles with κ-casein predominantly wrapped around the surface of the micelle. Casein fractions in the interior

TABLE 64.8 Composition and Some Characteristics of Caseins.

Casein	Approx.% of Total Casein	No. of Amino Acid Residues	Phosphate Groups	Approx. Mol. Wt.-Dalton	Isoelectric pH
α_{S1}-Casein	38	199	8	23,164	4.1
α_{S2}-Casein	10	207	10–13	25,388	4.1
β-Casein	35	209	5	23,983	4.5–5.3
κ-Casein	13	169	1	19,038	4.1–4.5

Source: Spreer (1998); Fox (2003); Otter (2003).

of micelle are sensitive to calcium and become insoluble in the presence of calcium. However, κ-casein is not sensitive to calcium and thereby keeps the micelles containing calcium-sensitive caseins intact and suspended in aqueous phase.

κ-Casein is a protein with hydrophilic carbohydrate moiety (sialic acid) that extends into aqueous phase. This arrangement further lends stability to the micelle. Casein micelles are stable at under most heating, homogenization, and other dairy processing conditions.

Caseins possess certain distinctive amino acid makeup which impacts their processing and functional properties. They are rich in apolar and hydrophobic amino acids, namely valine, leucine, isoleucine, phenylalanine, tyrosine, and proline. The apolar amino acids normally are insoluble in water, but their nature is balanced by phosphate groups so that caseins exhibit some solubility. Methionine and cysteine, the sulfur-containing amino acids are relatively low in caseins. This fact impacts their nutritional deficiency. On the other hand, the essential amino acid lysine content is high. In human diet, the high lysine content is helpful in complementing and balancing the low-lysine plant proteins. The ε-amino group of lysine present in caseins interacts with the aldehyde group of lactose at elevated temperature, leading to the formation of brown pigments (Maillard reaction). This also explains browning of heat-sterilized milk and nonfat dry milk during extended storage.

The high prolene content results in low α-helix and β-sheet in their secondary structure, giving them ability for more proteolytic degradation and enhanced digestion (Otter 2003).

Caseins possess limited secondary and tertiary structure. Accordingly, their molecular conformation is fairly flexible, and open. The polar and apolar amino acids in the primary structure of caseins contribute to hydrophilic and hydrophobic regions. This confers surface activity and contributes to emulsifying and foam-forming characteristics of caseins.

Caseins are very heat-stable under normal protein levels, environmental pH, and ionic concentrations. Moderate heat has little or no effect on casein molecules since they exist naturally in an open and extended state. However, heating of milk at elevated temperature for an appreciable length of time could result in hydrolytic cleavage of peptide and phosphate bond, which affects the stability of the complex, contributing to coagulation of milk.

Coagulation of milk is primarily a manifestation of micellar casein precipitation. This temperature-dependent phenomenon is critical in the manufacture of yogurt and fermented milks as well as in cheese making. The precipitation/coagulation mechanism consists of the following types:

ISOELECTRIC PRECIPITATION. Factors such as the pH strongly influence the electrostatic interactions in casein. Casein becomes insoluble and precipitates out when the milk is acidified and the pH is reduced to 4.6 at or above 20°C. At low temperature (4°C), no visible precipitation is observed. As the temperature is raised, coagulation is observed at or above 20°C. The proteins remaining in solution are whey proteins. The destabilization of micellar casein by added acid or by lactic acid produced during fermentation by lactic acid bacteria starts at pH 4.9 when colloidal calcium phosphate becomes soluble and changes to ionic form. As the pH reaches 4.6, calcium phosphate is cleaved in entirety from the micelle. At the same time, the isoelectric point of casein is reached and the micelle has no longer any charge to keep it suspended by repelling forces. The result is aggregation of casein micelles leading to dense coagulum. This type of coagulation is relevant in all fermented dairy products including cottage cheese and cream cheese. Many

textural attributes are controlled by the temperature, quiescent conditions, pH, and rate of acidification of milk.

RENNET COAGULATION. In cheese making, the mechanism of coagulation is not acid-based but is caused by enzymatic attack by acid proteinase, chymosin contained in rennet. This coagulation occurs at normal pH of milk. The specific cleavage of κ-casein molecule occurs at amino acid 105 (phenyl alanine) and 106 (methionine) to form para κ-casein and a macropeptide called glycomacropeptide (GMP). The GMP contains carbohydrate residues. Being hydrophilic, it is soluble and ends up in the whey fraction. Since micelle is stabilized by calcium-insensitive κ-casein, its hydrolysis by chymosin results in the exposure of calcium-sensitive α_s-caseins and β-casein to serum calcium, the overall effect is coagulum formation by aggregation of the micelles. Further hydrophobic interactions result in the expulsion of moisture from the coagulated micelles, causing syneresis and curd shrinkage. This coagulum is the basis of cheese curd formation. Para κ-casein is further degraded during cheese ripening to produce numerous flavor compounds and textural components.

POLYVALENT ION PRECIPITATION. Due to its disordered molecular structure, casein fractions also precipitate out in the presence of di- and polyvalent ions of various salts.

ALCOHOL PRECIPITATION. Casein micelles become unstable at 40% alcohol concentration at normal milk pH. At lower pH, the stability becomes even less and lower alcohol levels can precipitate milk. Dehydration of casein micelles appears to be major cause of this type of precipitation.

HEAT COAGULATION. Severe and extensive heating of milk can cleave the calcium phosphate complexes with casein micelle, resulting in destabilization, aggregation, and precipitation. Casein can withstand normal heating processes in dairy plants, interactions do occur with the whey proteins.

Among the minor caseins of milk, γ-casein is the C-terminal fragment of β-casein, a product of attack by natural proteolytic enzyme plasmin. The N-terminal residue is the proteose–peptone fraction. These hydrolysis products of β-casein occur at a range of 3–10% of the total casein content of milk. The stage of lactation and health status of the cow affect their concentration.

Peptides derived from caseins are biologically active and display significant extra nutritional attributes for maintaining normalcy of physiological functions in human subjects.

Whey/Serum Proteins. Whey proteins consist of β-lactoglobulin and α-lactalbumin, bovine serum albumin, immunoglobulins (mainly IgG1, IgG2, and IgM), lactoferrin, proteose-peptone, and a number of diverse enzymes. Table 64.9 shows some characteristics of whey proteins.

Compared to caseins, whey proteins have a relatively more ordered globular structure, which contains disulfide linkages. Accordingly, unlike caseins, they are soluble and not vulnerable to precipitation under acidic conditions or by polyvalent ions. Like other globular proteins, they are very heat labile and can be denatured at 90°C, resulting in gel formation. β-Lactoglobulin complexes with κ-casein in milk when subjected to rigorous heat treatment. All the whey proteins are superior in biological value as compared to caseins

TABLE 64.9 Composition and some Characteristics of Whey Proteins.

Whey Protein	Approx. % of Total Whey Protein	No. of Amino Acid Residues	Approx. Mol. Wt.-Dalton	Isoelectric pH
β-Lactoglobulin	7–12	162	18,277	5.2
α-Lactalbumin	2–5	123	14,175	5.1
Bovine serum albumin	0.7–1.3	582	69,000	4.8
Immunoglobulins	1.9–3.3	–	150,000–1,000,000	4.6–6.0
Proteose peptone	2–6	–	4,000–40,000	3.7

Source: Fox (2003); Otter (2003).

and compare with the quality of egg albumins. Major differences in the behavior of caseis and whey proteins are summarized in Table 64.10.

β-LACTOGLOBULIN. This major whey protein of milk displays the presence of four genetic variants. Besides the two genetic variants, namely A and B, variants, C and D have also been reported. β-Lactoglobulin is rich in sulfur amino acids, containing five cysteine residues. It exists as a dimmer linked by one to three disulfide bonds. It is a fairly heat labile protein. Heat treatment of 60°C results in partial denaturation. Differential scanning calorimetry results show peak maximum of denaturation at 80°C and formation of reactive sulfhydryl groups which can interact with κ-casein and/or α-lactalbumin by disulfide linkages. Further heating liberates hydrogen sulfide which is associated with "cooked" favor. β-Lactoglobulin stimulates lipolysis and generation of rancidity. It also acts as a carrier of vitamin A. The large numbers of lysine residues can result in lactosylation and accompanying changes in physical properties of the protein.

α-LACTALBUMIN. α-Lactalbumin is the major protein of human milk, but in cow milk it is second in preponderence to β-lactoglobulin. Three genetic variants are reported, but western cows contain variant B only. This protein is rich in tryptophan and sulfur amino acids cysteine and methionine. There are four disulfides in the molecule and it exists as a monomer. α-Lactalbumin has 54 amino acid linkages identical with the enzyme lysozyme. It is a glycoprotein as well as a metalloprotein. One mole of calcium is bound to each protein molecule, which confers heat stability on α-lactalbumin. This protein has been shown to possess a physiological role in the synthesis of lactose in the mammary gland. It is a component of lactose synthetase along with uridine diphosphate–galactosyl transferase, catalyzing the transfer of galactose to glucose to form lactose.

TABLE 64.10 Major Differences in Physical and Chemical Properties of Casein and Whey Protein.

Casein	Whey Protein
Strong hydrophobic regions	Both hydrophobic and hydrophilic regions
Phosphate residues	No phosphate residues
Little cysteine content	Both cysteine and cystine content
Random coil structure	Globular structure and helical structure
Very heat stable	Heat denatured and precipitates
Precipitates at pH 4.6	Soluble at pH 4.6
Precipitates with di- and polyvalent ions.	Relatively resistant to the ions

Source: Chandan (1997).

IMMUNOGLOBULINS. There are five major classes of immunologlobulins, namely, IgA, IgD, IgE, IgG, and IgM. Their concentration is very high (100 g/L) in first two to three milkings after calf birth, but falls to 0.6–1 g/L soon after. Immunoglobulins are antibodies synthesized in response to stimulation by specific antigens. These offer nonspecific humoral response to Gram-negative enteric and aerobic bacteria. Accordingly, they provide passive immune protection to the newly born calf. The basic structure of all Igs is similar, which is composed of two identical light chains (23,000 Daltons) and two identical heavy chains (53,000 Daltons). The four chains are joined together by disulfide bonds. The complete molecule has a molecular weight of about 180,000 Daltons. The antigenic sites are located at the $-NH_2$ terminal of the respective chain. Of the five-immunoglobulin classes, IgG is the predominant fraction of milk, comprising of about 90% of the total colostral immunoglobulins. Relatively smaller concentrations of IgM and IgA are also present in progressively decreasing amounts.

BOVINE SERUM ALBUMIN. As the name indicates, this protein originated from blood and during synthesis in the udder spills into milk. It is a large molecule with binding ability for fatty acids and metals.

LACTOFERRIN/LACTOTRANSFERRIN. This is a glycoprotein, which displays strong tendency to bind ionic iron due to the presence of two metal binding sites. The average lactoferrin content of 0.32 mg/mL has been found for cow milk. The molecular weight of lactoferrin varies between 73,700 and 74,000 Daltons. Lactoferrin display very strong chelating tendency for ionic iron and forms a salmon red color pigment. Lactoferrin is a single peptide chain, with two lobes, each of which is capable of binding iron. Iron free form of lactoferrin is known as apolactotransferrin, which is colorless in appearance. Lactoferrin displays strong inhibitory effect towards Gram-negative enteropathogenic bacteria by virtue of its ability to bind free ionic iron, which is essentially required for the growth of enteropathogenic microorganisms. Apart from the antibacterial effect in the gut of a calf, a nutritional role in iron metabolism has also been ascribed to lactoferrin.

Biologically Active Proteins and Peptides. A number of proteins and peptides derived from milk proteins have physiological activity. They are: (1) immunoglobulins, lactoperoxidase, lactoferrin, and folate-binding protein; (2) insulin-like growth factors (IGF-1 and IGF-2), mammary derived growth factors MDGF-I and MDGF-II), transforming growth factors ($TGF_{\alpha 1}$, $TGF_{\alpha 2}$, TGF_{β}), fibroblast growth factors, platelet-derived growth factors, bombesin, and bifidus factor; and (3) peptides derived from milk proteins, such as glycomacropeptides from κ-casein, phosphopeptides from caseins, caseinomorphins, immunomodulating peptides, platelet-modifying peptide, angiotensin-converting enzyme (ACE) inhibitor that lowers blood pressure, calmodulin-binding peptides, and bactericidal peptides from lactotransferrin (Otter 2003).

Milk Enzymes. Milk is a repository of a variety of enzymes. Over 60 indigenous enzymes have been reported in cows' milk. They are either associated with milk fat globule membrane (xanthine oxidase, sulfhydryl oxidase and γ-glutamyltransferase), with skim milk serum (catalase, superoxide dismutase), or with micelles of casein (plasmin and lipoprotein lipase). The partition and distribution of these enzymes is affected by processing and storage condition of milk. Other enzymes present are lactate dehydrogenase, malate dehydrogenase, lactoperoxodase, galactosyl transferase, alkaline phosphatase, phosphoprotein phosphatase, ribonuclease, lysozyme, fructose biphosphate

aldolase, and glucose phosphate isomerase. The enzymes in milk come either from the cow's udder (original enzymes) or from bacteria (bacterial enzymes). Several of the enzymes in milk are utilized for quality testing and control. Some of the enzymes, which are important from processing point of view, are described below:

ALKALINE PHOSPHATASE. This enzyme has assumed significance because of the association with the temperature at which it is inactivated and the temperature employed for pasteurization of milk. The basis of pasteurization is that the spore-forming pathogens, which may be present in milk, are completely destroyed by heat treatment designated in the pasteurization process. In turn, alkaline phosphatase activity is also destroyed by the pasteurization heat treatment. Thus, efficiently pasteurized milk should be safe from pathogens, and concomitantly, should not display any alkaline phosphatase activity. Contamination of pasteurized milk with raw milk can also be detected by positive phosphatase activity in milk. Alkaline phosphatase is distributed through milk. Its concentration is higher in the cream fraction. The optimum pH for the action on alkaline phosphatase on p-nitrophenylphosphate is 9.5. The K_m value for this substrate is 6.6×10^{-4} M for skim milk enzymes, whereas for the cream alkaline phosphatase the corresponding value is 3.6×10^{-4} M.

LIPOPROTEIN LIPASE. This enzyme brings about hydrolytic cleavage of glycerides, liberating free fatty acids and glycerol. The volatile short chain free fatty acids generate undesirable rancid flavor in milk. Thus, the activity of this enzyme can result in rancid flavor defects in dairy products. Lipase is activated by homogenization of fat globule membrane in raw milk. Similarly, lipase can degrade milk fat and develop off-flavor in short period of storage, if raw milk accidentally gets mixed with homogenized milk. The optimum pH for the enzymatic activity ranges from 8.4 to 9.0, while optimum temperature for enzymatic activity is 37°C. Sodium chloride and magnesium chloride have stimulatory effect on these enzymes whereas calcium chloride and manganese chloride have inhibitory effect. Residual activity of lipase remaining in processed milk or milk products tends to reduce their shelf life.

PROTEASE/PLASMIN. This enzyme is responsible for the hydrolytic degradation of proteins. The optimum activity is observed at a temperature of 37°C and a pH of 8.0. Nearly 82% of proteolytic activity is lost when milk is pasteurized. Native proteases of milk are more heat labile compared to the microbial proteases, which tend to survive even UHT processing treatment. Residual proteolytic activity in processed milk and milk products leads to decrease in shelf-life.

LACTOPEROXIDASE. This enzyme catalyzes oxidation of substrate in the presence of an oxygen donor such as hydrogen peroxide. It displays optimum activity at pH of 6.0 and is stable over a wide pH range of 5–10. This enzyme has gained significance in view of its supportive role for the preservation of raw milk employing LP-system under ambient conditions.

LYSOZYME. This is a relatively small, single peptide chain protein. The variant found in bovine milk has 129–130 amino acid residues, with molecular weight of 14,000. The lysozyme cleaves the glycosidic linkage between N-acetylmuramic acid and N-acetylglucosamine of bacterial cell wall. Gram-positive bacteria are generally more susceptible because they have a simpler cell wall providing greater accessibility of the

substrate compared to the Gram-negative bacteria. Cow milk contains about 13 μg/100 mL of lysozyme. More recently, emphasis has been focused on the antibacterial role of lysozyme as a natural defense in milk. During mastitis, lysozyme levels in milk tend to increase considerably, being in the range of 100–200 μg/100 mL. It has also been suggested that lysozyme may have an indirect effect on the defense systems as an immunomodulator through the stimulation of break-down products of the peptidoglucan on the immunosystem.

Functional Attributes of Major Milk Proteins. Milk proteins are used in various foods to impart desirable effects. Table 64.11 shows such characteristics of milk proteins which are helpful in their use as functional ingredients.

64.5.1.4 Lactose. The major carbohydrate of milk, lactose monohydrate, ranges from 4.8% to 5.2%. Lactose content of milk is relatively constant. In colostrum and mastitic milk, its concentration is significantly lower. It forms 52% of milk solids-not-fat and 34% whey protein concentrate, and 70% of whey solids. It a disaccharide of one residue each of D-glucose and D-galactose. Structurally, lactose is 4-O-β-D-galactpyranosyl-D-glucopyranose. Fresh milk contains small amounts of glucose (100 mg/100 mL), galactose (100 mg/100 mL) and oligisaccharides (10 mg/100 mL). It is a reducing sugar and extensive heating of milk results in Maillard reaction between lactose and proteins creating brown pigments and brownish color of milk.

In isolated form, lactose exists in either of the two crystalline forms, α-hydrate and anhydrous-β or as amorphous "glass" mixture of α- and β-lactose. In solution both the forms exist in equilibrium with a ratio of (α : β) 1.68 at 20°C. Lactose has an asymmetric carbon and therefore displays optical activity. Lactose anomers rotate plane polarized light and their concentration can be assayed by polarimetric measurements. The α-lactose anomer is more dextrorotatory than β-lactose. If lactose crystallizes from solution like milk or whey below 93.5°C, α-lactose is usually formed, while above 93.5°C, β-lactose

TABLE 64.11 Functional Properties of Milk Proteins.

Functionality	Casein or Caseinates	Whey Proteins
Water binding	Very high, minimum at pH 4.6	Water-binding capacity increases with denaturation of the protein
Solubility	Insoluble at pH 4.6	Soluble at all pH levels. If denatured, insoluble at pH 5
Viscosity	High at or above pH 4.6	Low for native protein. Higher, if denatured
Gelation	No thermal gelation except in the presence of Ca^{2+}. Micelles gel with rennin	Heat gelation at 70°C (158°F) or higher and influenced by pH and salts
Emulsification	Excellent at neutral and basic pH	Good except at pH 4–5, if heat denatured
Foaming	Good overrun. K-Casein best followed by β- and α_{s1}-caseins Poor foam stability	Good foam/overrun β-lactoglobulin better than α-lactalbumin
Flavor binding	Good	Retention varies with degree of denaturation

Source: Chandan (1997); Fox (2003).

usually crystallizes. During crystallization, the β-form mutarotates to α-lactose. Crystals of α-lactose monohydrate are shaped like tomahawk and other shapes arise as a result of co-crystallization on the face of lactose crystals. The rate of crystallization, size and shape of lactose crystals depend on the degree of super saturation of lactose solution and the inhibitor (β-form) level.

The α-form is less soluble (70 g/L at 15°C) than the β-form. Crystallization of lactose, when milk is concentrated is of importance in regard to the texture. An equilibrium mixture of α- and β-lactose, formed by mutarotation, exhibits a solubility of about 155 g/L at 10°C and 119 g/L of water at 0°C. The relatively poor solubility at low temperatures (4°C or below) contributes to sandy texture in high milk solids ice cream, processed cheese products and condensed milk products. As a general rule, a concentration of lactose exceeding 13 g/100 mL water in a dairy product tends to promote crystallization of α-lactose monohydrate and accompanying sandy texture defect. In the manufacture of non-hygroscopic dry milk and whey, lactose crystallization plays an important role. In rapid drying conditions, lactose glass (amorphous lactose) is formed. This form of lactose is very hygroscopic and causes caking in dried products containing moisture levels of 8% or more. Under such conditions, the conversion of lactose glass to α-lactose monohydrate crystals is responsible for binding powder particles together as a "cake."

In sweetening power, lactose is only 16–33% as sweet as sucrose. This makes lactose uniquely suitable for certain food applications. Toppings icing and various types of fillings are examples of use where its inclusion in the formulations can improve the quality. The pharmaceutical industry has used lactose for many years for tablet or pill formation. Being a reducing sugar, it reacts with proteins to form a highly flavored golden brown substance, commonly found on the crust of baked foods. Lactose contributes significantly to the flavor, texture, appearance, shelf-life, and toasting qualities of baked foods.

A compound formed from lactose in heated milk products is lactulose. It stimulates the growth of *Bifidobacterium bifidum* and is thus beneficial in establishing useful microflora in the gut.

The role of lactose in yogurt and fermented milks is extremely important because the culture nutritionally requires it as a substrate for growth. It is a source of carbon and after fermentation about 30% of the lactose content is converted to lactic acid. Lactose is easily hydrolyzed by β-D-galactosidase or lactase enzyme of the culture to glucose and galactose. Glucose is readily metabolized by the Embden-Meyerhof-Parnas pathway, while galactose tends to accumulate. One molecule of lactose gives one molecule of galactose and two molecules of lactic acid. Energy is generated in this reaction. The acid production lowers the pH enough so that the fermented food is safe from most pathogens. The shelf-life of fermented milks is significantly increased because many spoilage organisms cannot grow at their low pH.

Digestion of lactose presents a problem in some individuals. They lack the enzyme β-D-galactosidase in their gastrointestinal tract. Consequently, dietary lactose is not hydrolyzed and it reaches the colon intact where it is metabolized by colonic bacteria forming gases like methane and hydrogen. It leads to discomfort caused by bloating and diarrhea. This lactose malabsorption is alleviated by yogurt containing live cultures, because the culture furnishes the lactose hydrolyzing enzyme and normal digestion pattern is restored.

64.5.1.5 Minerals. Average normal milk is considered to contain 0.70% ash and this amount represents a salt content of about 0.90%. The percentage of salt and ash in milk

TABLE 64.12 Typical Mineral Content of Cows' Milk.

Major Mineral	Mean (mg/100 mL)	Range (mg/100 mL)
Calcium-total	121	114–130
Calcium-ionic	8	6–16
Citrate	181	171–198
Chloride	100	90–110
Magnesium	12	9–14
Phosphorus, inorganic	65	53–72
Potassium	144	116–176
Sodium	58	35–90
Trace Elements	**μg/100 g of Milk**	**μg/100 g of Milk**
Boron	27	–
Chromium	1	0.8–1.3
Cobalt	0.1	0.05–0.13
Copper	20	10–60
Fluoride	12	3–22
Iodine	26	–
Iron	45	30–60
Manganese	3	2–5
Molybdenum	7	2–12
Nickel	2.5	0–5
Selenium	12	5–67
Silicon	260	75–700
Zinc	390	200–600

Source: Swaisgood (1996); Fox (2003).

varies with the breed, feed, season, and stage of lactation and disease. The white residue after incineration of a given weight of milk is used as a measure of the mineral content of milk. Ash content is not identical to milk mineral level because of decomposition and volatilization of certain minerals due to heat. The ash contains substances derived from both the organic and inorganic compounds in the milk. The CO_2 of the carbonates is formed mostly from the organic components; the SO_3 of the sulfates is considered to be a decomposition product of the proteins. Part of the P_2O_5 arises from the casein, since this protein contains phosphorous equivalent to about 1.62% P_2O_5. Citric acid is completely lost. Chloride is partly lost (45–50%) by the high temperature employed for ashing. This loss can be minimized by keeping the temperature below 600°C. The mineral content of milk is given in Table 64.12.

Mineral make-up of milk is crucial to the stability of the physico-chemical equilibrium in milk. The minerals of milk exist in colloidal and soluble form. Table 64.13 gives approximate phase composition of the minerals.

They are present in a complex equilibrium consisting of colloidal state and soluble state. The soluble state exists in both ionic and nonionic form, and their ratio is influenced by pH of milk. Their concentration is less than 1% in milk but the technological behavior of milk is impacted a great deal by them. For instance, the following characteristics are influenced:

1. Heat stability and alcohol coagulation of raw milk.
2. Preparation, quality and storage stability of products like concentrated/condensed, evaporated milk products.
3. Clumping of fat globules upon homogenization of cream.

TABLE 64.13 Partition of Major Minerals in Colloidal and Solution Phases.

	Percent of Total Mineral as	
Major Mineral	Colloidal	Dissolved
Calcium	67	33
Magnesium	36	64
Sodium	4	96
Potassium	6	94
Phosphate	55	45
Citrate	6	94
Chloride	0	100
Sulfate	0	100

4. The calcium content of milk influences the firmness of curd during cheese making and the viscosity of fermented milks. From nutritional standpoint, milk is an excellent source of calcium and phosphorus. Their ratio in milk is optimal for bone formation and bone health.

Sodium, potassium, and chloride are almost completely (95–96%) present in true solution and in ionic form and therefore diffuse freely across the membrane during ultrafiltration and electrodialysis of milk and whey. Calcium and magnesium, phosphate and citrate are partly in solution and partly in colloidal suspension, depending on the pH of milk. Approximately 20–30% of diffusible Ca and Mg exist as free ions and the remainder as salts of citrate and phosphate. As the pH of milk drops in the manufacture of yogurt and fermented milks, the colloidal form is converted progressively to the ionic form. At pH 4.4 most of the minerals are in ionic, soluble and diffusible form.

64.5.2 Vitamins and Some Other Minor Constituents

The concentration of fat soluble vitamins A, D, E, and K, and water soluble vitamins B and C, and minor constituents of milk are given in Table 64.14.

Milk contains both fat soluble (A, D, E, and K) and several water soluble vitamins. In the production of low fat and skim milk, the fat soluble vitamins get concentrated in the cream fraction. Whole milk is a good source of vitamin A but the separation process leads to low vitamin A content in low fat and skim milk. The FDA regulations require fortification of low fat and skim milk to restore and to make the vitamin A content of low fat and skim milk equivalent to that of whole milk. The regulations require 2000 IU of vitamin A per quart of milk. The objective is to insure essentially the same dietary vitamin A contribution of all fluid milk beverages. Natural vitamin A activity in milk is due to retinol and the pigment β-carotene. Their level as well as those of vitamin D and E varies in milk according to the season and feed profile. Vitamin D is important in bone health and vitamin E in an antioxidant. Vitamin K is present in milk but its dietary nutritional role is minor.

Milk is an important source of dietary B vitamins. They are stable to various heating and processing conditions milk is normally subjected to. Riboflavin is vulnerable to light (wavelength <610 nm), generating sunlight flavor defect in milk. Ascorbic acid

TABLE 64.14 Vitamins and some Minor Components of Milk.

Vitamins	Per 100 g of Milk
A	40 μg RE
D	4 IU
E	100 μg
K	5 μg
B_1	45 μg
B_2	175 μg
Niacin	90 μg
B_6	50 μg
Pantothenic acid	350 μg
Biotin	3.5 μg
Folic acid	5.5 μg
B_{12}	0.45 μg
C	2 mg
Nonprotein Nitrogen Compounds	
Total NPN	23–31 mg
Urea N	8–13 mg
Creatine N	0.6–2 mg
Uric acid N	0.5–0.8 mg
Orotic acid N	1.2 mg
Peptides N	3.2 mg
Ammonia N	4–5 mg
Choline	4–28 mg
Carnitine	1–1.7 mg
N-acetyl neuraminic acid	12–27 mg
Miscellaneous Compounds	
Nucleic acids and nucleotides	56 mg
Phosphoric esters	30 mg
Ethanol	0.3 mg
Lactic acid	3.5–10 mg
Citric acid	175 mg
Acetic acid	0.3–5 mg
Formic acid	1–8.5 mg

Source: Goff and Hill (1992).

(vitamin C) content of milk is very low and not significant. Also, it is inactivated by heat processing.

As shown in Table 64.14, some nonprotein nitrogen compounds and several miscellaneous compounds are also detected in milk.

64.6 PHYSICAL CHARACTERISTICS OF MILK

64.6.1 Optical Properties

64.6.1.1 Color. The color and appearance of milk has significance because the consumers perceive it as parameter of quality. The opaque, white, or turbid color of milk is due to scattering of light by the dispersed phase of fat globules, casein micelles, and the colloidal calcium phosphate. The intensity of color is directly proportional to the size and number of these particles. The smaller particles scatter light of shorter wavelength. The creamy color of whole milk is due to its β-carotene content. Some breeds (e.g., Guernsey cows) have

more of this pigment and their color is yellowish/"golden." In cases of goat milk and water buffalo milk, the pigment content is very low. β-Carotene is a precursor of vitamin A and in milk of goats and water buffaloes, it is inherently converted to vitamin A. Accordingly, their milk has white color as opposed to creamy color of cows' milk.

Extended heating imparts a slightly brown color to milk as a result of Maillard's reaction between lactose and proteins.

Homogenization increases the number and total volume of fat globules. This results in whiter color of homogenized products than their unhomogenized counterparts. Lack of fat globules and water-soluble pigment riboflavin produces a bluish green tint in skim milk. In the absence of fat globules, light scattering is primarily by casein micelles which scatter more blue (short wave lengths of light) than the red. The color thus becomes distinctly green fluorescence in whey after removal of casein particles from skim milk. Yellow color of cow milk fat in butter and cream is due to the presence of fat-soluble pigments carotene and xanthophyll.

64.6.1.2 Refractivity. The refraction of light by a solution is a function of the molecular concentration of the solute in solution. Each solute maintains its own refractivity, and the refractive index of a mixture is that of the total of the refractive indices of the substances plus that of the solvent. The components of milk contributing to its refractive index in descending order of importance are water, proteins, lactose, and minor constituents. Specific refractive increments in water at wavelength 589.3 nm and 20°C, in mL/g, for casein complex, whey proteins, lactose, and other dissolved substances are 0.207, 0.187, 0.140, and 0.170, respectively. The fat globules do not contribute to refractive index of milk because refraction occurs at the interface of plasma and air.

Refractive index of a substance varies with the wavelength of the light and temperature of measurement. It is generally measured at 20°C with D line of sodium spectrum (wave length 589.3 nm) and represented as n_D^{20}. The value of n_D^{20} of cow milk generally falls in the range 1.3440–1.3485. Refractive index of human and goat milks have slightly higher value. The refractive index of milk fat ranges from 1.4537 to 1.4552 at 40°C and is used for verification of its authenticity.

64.6.2 Flavor

Taste and aroma are critical to the assessment of milk. Flavor constitutes a critical criterion of quality for the consumer. It is a sensory property in which odor and taste interact. The sweet taste of lactose is balanced against the salty taste of chloride, and both are somewhat moderated by proteins. This balance is maintained over a fairly wide range of milk composition even when the chloride ion level varies from 0.06% to 0.12%. Saltiness can be detected by sensory tests in samples containing 0.12% or more of chloride ions and becomes marked in samples containing 0.15%. Some workers attribute the characteristic rich flavor of dairy products to the lactones, methylketones, certain aldehydes, dimethyl sulfide, and certain short-chain fatty acids.

Although milk has a clean, pleasantly sweet flavor, it is quite bland, and therefore, any off-flavors are readily discernible. Off flavors result when the balance of flavor compounds is altered due to the microbiological activity or processing treatments, or chemical or biochemical reactions. The fat globules have a large surface area and tend to adsorb aromatic odors (for example, onion, and garlic) readily.

Some off flavors in milk are shown in Tables 64.15 to 64.18.

TABLE 64.15 Off-Flavors in Milk Caused by Absorption from the Feed and Environment.

Off-flavor	Description	Possible Cause
Feed	Aromatic, onion, garlic	Cows fed 0.5–3 h before milking
Cowy	Chemical after-taste, cow's breath odor	Cows with ketosis/acetonemia
Barny	Unclean, reminiscent of barn, silage	Poor ventilation, build up of aromatic silage/barn odors

TABLE 64.16 Milk Off-Flavors of Microbiological Origin.

Off-Flavor	Description	Possible Causes
Malty	Grape nut-like, caramelized, burnt	Unsanitary equipment, insufficient cooling and storage at $>10^{\circ}$C.
Bitter/unclean	Musty, spoiled, stale, dirty, bitter	Exposure to warm temperature, dirty utensils, weeds
Fruity/fermented	Odor resembling fruits like apple/pineapple	Old milk, too long storage of raw milk
Sour	Tingling acidic taste	Growth of lactic and other organisms

TABLE 64.17 Off-Flavors in Milk of Biochemical Origin.

Off-Flavor	Description	Possible Cause
Rancid	Bitter, soapy, foul odor, unclean	Homogenized raw milk stored too long, mixture of pasteurized and raw milk, raw milk agitated vigorously
Oxidized/light induced	Medicinal chemical taste, reminiscent of burnt feather or tallow	Milk in transparent plastic/glass bottles exposed to sunlight or UV light in refrigerated cases

64.6.3 Acidity and pH

Freshly drawn milk shows certain acidity as determined by titration with an alkali (sodium hydroxide) in the presence of an indicator phenolphthalein (equivalent to pH 8.3). This acidity, also called titratable acidity, as determined by titration, is known as "natural" or "apparent" acidity. It is caused by the presence of casein, acid-phosphates, citrates, and so on, in milk. The natural acidity of individual milk varies considerably depending on species, breed, individuality, stage of lactation, physiological condition of

TABLE 64.18 Off-Flavors Arising from Processing Conditions.

Off-Flavor	Description	Possible Cause
Cooked	Sulfur-like odor, caramelized, scorched	Too high pasteurization temperature and holding time too long, Excessive heat treatment
Foreign	Non-milk like odor/flavor	Contamination with sanitizers, cleaning compounds
Flat	Watery	Too low milk solids, watered milk

the udder, and so on, but the natural acidity of fresh, herd/pooled milk is much more uniform. The higher the solids-not-fat content in milk, the higher the natural acidity and vice versa. The tiratable acidity of individual cow milk varies from 0.12% to 0.18%, but in commercial pooled milk the range is only 0.14–0.16%. "Developed" or "real" acidity is due to lactic acid, formed as a result of bacterial action on lactose in the milk. Hence, the titratable acidity of stored milk is equal to the sum of natural acidity and developed acidity. The titratable acidity is usually expressed as a "percentage of lactic acid." The higher the serum solids, the higher are the titratable acidity, but pH remains relatively the same. The titratable acidity (or pH measurement) is a critical parameter in yogurt and fermented milk production. It determines the end point of fermentation process. Measuring pH is preferable because unlike titratable acidity, it does not vary with the total milk solids-not-fat in yogurt mix.

The pH of normal, fresh, sweet milk usually varies between 6.6 and 6.8. Higher pH values for fresh milk indicate udder infection (mastitis) and lower values, bacterial action. Skimming and dilution with water raise the pH of milk while sterilization usually lowers it.

64.6.4 Buffering Capacity

The pH is a measure of acidity or inverse of the logarithm of the hydrogen ion concentration in milk. The relationship of hydrogen ion concentration and pH is shown by the following equation. A weak acid (HA) dissociates as follows:

$$K_a = \frac{(H^+)(A^-)}{(HA)}$$

where, K_a is dissociation constant, (H^+) is hydrogen ion concentration, and (A^-) is the concentration of the anion and (HA) is the concentration of the acid HA.

$$pH = \log\frac{1}{(H^+)} = pK_a + \log\frac{(A^-)}{(HA)}$$

where pK_a equals to $-\log_{10} pK_a$. When pH equals pK_a, the weak acid is 50% dissociated and the buffering capacity is maximum. Proteins contain many basic and acid groups in their molecule. Generally, their maximum buffering capacity is at their isoelectric point.

Milk displays innate ability to resist the changes in the pH or buffering capacity (dB/dpH). This is mainly due to the presence of amino acid residues of caseins and whey proteins, and colloidal salts (calcium phosphate complex, citrates, etc.). Caseins display maximum buffering capacity at their isoelectric pH of 4.6 and phosphates around pH 7.0. Whey proteins show maximum buffering capacity at pH 4–5. The buffer index of milk is defined as the amount of acid or alkali (moles/L) required changing pH of one liter of milk by one unit.

Buffering capacity has some significance in the survival of live cultures in the stomach where high acid conditions are deleterious to the survival of yogurt cultures. Since yogurt pH is close to their isoelectric point, the milk proteins of yogurt exercise maximum buffering capacity. Accordingly, the impact of acidic conditions on the culture cells is somewhat moderated for better survival rates in the stomach.

64.6.5 Electricochemical Properties

64.6.5.1 Oxidation Reduction Potential. The oxidation-reduction (E_h) potential of milk is expressed in volts. It is measured relative to the potential of the standard hydrogen electrode which is assigned 0 volt at pH 0. It is due to the presence of several soluble constituents capable of yielding or accepting electrons. In milk E_h is controlled by the factors such as dissolved oxygen, ascorbic acid, riboflavin, cystine–cysteine transformation and pH value. Fresh cow milk displays values of +0.2 to +0.3 volt at 30°C. It is due largely to dissolved oxygen, ascorbic acid and riboflavin. Bacterial growth reduces the oxygen tension. Methylene blue reduction test, used for assessing the microbial quality of milk is based on this phenomenon. The ascorbic acid oxidation in stored milk leads to the formation of singlet oxygen, which in turn is involved in lipid oxidative deterioration. Riboflavin in milk exposed to light near 450 nm, assists in photooxidation of methionine residues of whey proteins to produce methional, the principal cause of "sunlight" flavor defect. Heating of milk increases the reducing capacity of milk and above 70°C also causes noticeable decrease in the Eh due to liberation of −SH groups from whey proteins. The increase in reducing capacity of yogurt mix after heat treatment is significant in promoting the growth of yogurt bacteria which are microaerophilic in nature.

64.6.5.2 Electrical Conductivity. Current passes through the milk by virtue of the activity of its ionic mineral constituents, of which chloride ions carry 60–68% of the current. There is, therefore, a close correlation between the electrical conductivity of milk and its chloride content. The specific electrical conductivity of milk at 25°C ranges between 0.004 ohm/cm and 0.0055 ohm/cm, corresponding to that of approximately 0.25% NaCl solution (w/w). Higher values usually represent mastitic infections. Sodium, potassium, and chloride ions are the major contributors to electrical conductance of milk. Whey and permeate from ultrafiltration have higher conductivity than skim milk. The presence of fat tends to decrease the specific conductance. Conductivity of milk may be used to detect added neutralizers.

The development of acidity by bacterial action during fermentation of milk increases its conductance because of conversion of calcium and magnesium to ionic forms. Thus measuring their electrical conductance can follow the progress of fermentation during manufacture of yogurt and other fermented dairy products. Electrical conductance can also be used to follow demineralization of whey leading to loss of ionic minerals, during manufacture of whey protein concentrates. Electrical conductance is directly proportional to temperature. Conductance of milk increases by about 0.0001 ohm/cm/C.

64.6.6 Thermal Properties

64.6.6.1 Thermal Expansion. When warmed the volume of milk increases which affects the design considerations for storage and flow rates through processing treatments. The coefficient of thermal expansion of fresh milk (4% fat, 8.95% SNF) is approximately 0.335 $cm^3/kg/C$ at temperature range of 5–40°C.

64.6.6.2 Heat Capacity. Heat capacity of milk and milk products, a function of total solids of the sample, decreases with their increasing contents. Heat capacity is expressed as (SI units). It equates to 1/4186 cal/g/°C in cgs units. Heat capacity of whole milk, skim milk, whey, milk fat, 40% cream, and dried skim powder at 15–20°C is 0.93, 0.95, 0.97,

0.52, 0.68, 0.30 cal/g/°C, respectively. Heat capacity increases linearly with increase in temperature in skim milk from 3906 J/kg/K at 50°C to 4218 J/kg/K at 140°C, according to the following equation (Goff and Hill 1993):

$$\text{Heat capacity} = 2.814 \times \text{Temperature in } ^\circ\text{C} + 3824$$

The heat capacity of milk and cream depends strongly upon fat content. Milk fat has a heat capacity of 2177 J/kg/K. The heat of fusion is 8.37 J/g. The heat capacity of milk in the range of 50–140°C can be estimated according to the equation:

$$\text{Heat capacity of milk} = 2.976 \times \text{Temperature in } ^\circ\text{C} + 3692$$

64.6.6.3 Specific Heat. Specific heat is the ratio between the amount of heat necessary to raise a given weight of a substance to a specified temperature and the amount of heat necessary to raise an equal weight of water to the same temperature. It is nearly identical to heat capacity figure as the heat capacity of water (1 cal/g/°C or 4186 J/kg/K) is fairly constant over the range of 0–100°C. It is important in processing for determining the amount of heat or refrigeration necessary to change the temperature of milk.

64.6.6.4 Thermal Conductivity. Thermal conductivity determines how fast milk is cooled or heated. It is the rate of heat transfer by conduction in J/m/s/K. Thermal conductivity increases as temperature increases. It decreases as the concentration level increases and for a given temperature and concentration, the higher the fat content, the lower the thermal conductivity. Thermal conductivity of proteins, carbohydrates, fat, milk, solids, and water at 30°C is 0.20, 0.245, 0.18, 0.26, 0.573, kcal/m/h/C, respectively. The thermal conductivity for milk at 37°C is 193 J/m/s/K, and 223 J/m/s/K at 80°C. There is a marked decrease in the thermal conductivity with increase in either fat or total solids.

64.6.6.5 Effects of Heat. Dairy plants routinely use heat processes to make milk safe from pathogenic organisms and to extend the shelf-life of milk and milk products. The pasteurization and sterilization temperatures and holding times employed in such treatments have profound effects on milk proteins, enzymes, fat globule membrane, some vitamins, and physical state of minerals and other constituents. Caseins of milk are relatively stable to moderate heating regimes under conditions of normal pH and ionic balance. The serum proteins are globular proteins. They are more prone to denaturation to heat. At 60–65°C, β-lactoglobulin molecules begins to uncoil themselves and start interaction with κ-casein located in casein micelle forming disulfide linkages. The denaturation process is complete at 90–95°C when milk is held for 5 min. Under this heat treatment, α-lactalbumin is relatively less vulnerable to heat, undergoing reversible denaturation. However, the immnnoglobulins are fully denatured. In the manufacture of yogurt, this heating treatment is beneficial in increasing water-holding capacity and in reducing syneresis of the coagulum. Also, the resultant viscosity increase assists in optimizing the texture of yogurt. High heat treatment is deleterious to rennin curd formation, and should be avoided.

Normal pasteurization treatment causes “cream plug phenomenon” in which some fat globules break down to free fat that sticks to other fat globules giving rise to the plug. On homogenization, the plug is broken down. Exposure to higher temperatures (>135°C),

results in partial aggregation of proteins of milk fat globule membrane and a more dense membrane that is less permeable.

Severe heat treatment above 100°C gives rise to brown pigments (melanoidin polymers) in milk. The Maillard reaction between the ε-amino group of lysine residue of proteins and carbonyl group of lactose gives brown color to milk. Such heat treatment also results in nutritional compromise. Cooked flavor results from the production of sulfhydryl groups arising from the breakdown of disulfide linkages.

64.6.6.6 Heat Stability. In the manufacture of certain high heat treated/concentrated milk products, heat stability of milk plays a significant role. A number of factors interact in a complex manner, which ultimately determines the heat coagulation of milk. On the basis of significant findings the role of various interacting factors may be summarized as follows.

Protein Composition. Various genetic variants of the casein fractions display variable heat stability. The heat coagulation of milk is related to the ratio between κ-casein and β-lactoglobulin. Higher heat coagulation temperature is observed at higher levels of β-lactoglobulin.

Mineral Balance. The heat stability of milk is mainly determined by the make up of proteins as well as the relative concentration of various salts present in colloidal and ionic states. The molar ratios between various cations and anions (both monovalent/polyvalent) strongly impact the physical equilibria of milk and the heat stability. Heat stability is maximum at the optimum salt equilibria defined by the relative concentration of Ca^{2+}, Mg^{2+}, $citrate^{3-}$, and $phosphates^{3-}$.

The molar ratio between cations and anions mainly determines whether milk will be stable at certain temperature and pH employed for processing. When milk is heated, salts of calcium and magnesium display an inverse solubility curve manifested by progressive transition of calcium and manganese from colloidal state to the ionic state. However, the solubility of the salts of sodium and potassium increases with the rise in processing temperature.

pH. The pH plays a critical role in determining the heat stability of milk. The pH effects both the molecular disassociation of casein components and formation of aggregated protein complexes through protein–protein interactions. Further, pH strongly affects the salt equilibrium between the colloidal and ionic states of the minerals of milk. Maximum heat stability is observed between pH 6.6–6.8.

Concentration of Milk Solids. In general, the heat stability of milk decreases progressively as milk is concentrated to higher levels of total solids. This is accompanied by concomitant shift of salt from ionic state to the colloidal state as well as drop in the pH values.

Homogenization. Although fat itself does not affect heat stability of milk, homogenization of milk brings about certain significant changes in the physical equilibria of milk. During homogenization of milk, the original fat globule is disrupted and surface area increases by many folds. Resurfacing of the newly formed fat globules take place instantly, predominantly by the adsorption of micellar casein. A shift in the colloidal

state due to the adsorption of caseins affects the equilibria between the colloidal and ionic states, which ultimately reduces the heat stability, although only marginally.

64.6.7 Density and Specific Gravity

64.6.7.1 The Density. The density of milk (mass/volume) is the sum total of the densities of its constituents, their concentration, and state at a particular temperature. The density of milk is a useful parameter to convert volumetric measurements to gravimetric and vice versa. Milk is purchased on weight basis and is sold in volumetric packages. Yogurt is sold in avoirdupois/weight units, while fermented milks are packaged in volumetric unit. Most of the dairy plants process milk and other products in gallons, a volumetric measure. Density is also useful in estimating degree of concentration during condensed milk manufacture by a simple hydrometer reading.

Milk density at 20°C ranges from 1.027 to 1.033 with an average of 1.030 g/cm^{-3}. Accordingly, the weight of 1 L of milk would range from 1.027 kg to 1.033 kg. The density of milk at 20°C can be calculated according to the following formula.

$$d_{20^\circ C} = \frac{100}{F/0.93 + SNF/1.608 + Water\%} g/cm^{-3}$$

where, d represents density, F = %fat, SNF = %solids-not-fat, and Water% = 100-F-SNF.

The density of some fluid milk products is given in Table 64.19.

64.6.7.2 The Specific Gravity. The specific gravity of milk is the ratio of density of milk to that of water at a given temperature. Yogurt mix and other dairy mixes containing sugar and added milk solids exhibit higher density and specific gravity than milk. For instance, the specific gravity of ice cream mix is 1.0544–1.1232, while that of fresh whole milk lies in the range 1.030–1.035, with an average of 1.032. Milk fat, milk solids-not-fat, skim milk, and evaporated whole milk, at 15.5°C, have specific gravity of 0.93, 1.614, 1.036, and 1.066, respectively. The specific gravity of milk is influenced by the proportion of its constituents, each of which has a different specific gravity approximately, as follows: water, 1.000; fat, 0.930; protein, 1.346; lactose, 1.666; salts, 4.120; and solids-not-fat, 1.616. As the milk fat is the lightest constituent, the more there is of it, the lower the specific gravity will be and vice versa. Determination of density of milk is

TABLE 64.19 Density of Fluid Milk Products at Various Temperatures.

	Density (kg cm^{-3}) at Various Temperatures			
Product	4.4°C	10°C	20°C	38.9°C
Raw milk, 4% fat	1.035	1.033	1.030	1.023
Homogenized milk, 3.6% fat	1.033	1.032	1.029	1.022
Skim milk, 8.9% SNF	1.036	1.035	1.033	1.0026
Half and half, 12.25% fat	1.027	1.025	1.020	1.010
Light cream, 20% fat	1.021	1.018	1.012	1.000
Whipping cream, 36.6% fat	1.008	1.005	0.994	0.978

Source: Goff and Hill (1993).

carried out by first warming the milk to 40°C to allow melting of fat and then adjusting the temperature down to desired working temperature.

The percentage of total solids or SNF in milk can be estimated by the following formula:

$$\%\text{TS} = 0.25\text{D} + 1.22\text{F} + 0.72$$
$$\%\text{SNF} = 0.25\text{D} + 0.22\text{F} + 0.72$$

where, D = 1000 (d − 1), d = density of sample of milk at 20°C, F = fat percentage of sample.

64.6.8 Viscosity

The viscosity of milk and cream creates the impression of "richness" to the consumer. From an organoleptic standpoint, viscosity contributes to mouth feel and flavor release. Fluidity is the inverse of viscosity. It has a bearing on fat separation/creaming, rate of heat transfer and flow conditions during processing of milk. Assuming laminar flow with parallel stream lines, viscosity may be defined as the ratio of shearing stress (force per unit area) to shear rate (velocity difference divided by distance, in reciprocal seconds). In dairy industry, the common units are centipoise (cp) or (poise $\times 10^{-2}$).

Viscosity of milk and dairy products depends on the temperature and on concentration and state of casein micelles and fat globules. Representative values at 20°C are whole milk: 1.9 cp, skim milk 1.5 cp and whey 1.2 cp. Viscosity of milk and cream increases with homogenization and the increase is proportional to the homogenization pressure. Increase in viscosity can be attributed to the fine state of fat globules and formation of a coat of plasma proteins on them.

The casein micelles of milk contribute more to the viscosity of milk than any other constituent. Viscosity varies not only with changes in physical nature of fat but also with the hydration of proteins. Alterations in the size of any dispersed constituents result in viscosity changes. The fat contributes less than caseins but more than whey proteins. When fat globules are greatly subdivided by homogenization, an increase in viscosity is observed. The viscosity of skim milk decreases on heating to 62°C after which it increases apparently due to changes in protein hydration. An increase in temperature causes a marked reduction in viscosity. For example at 20°C, milk is about half as viscous as at 0°C, and at 40°C, is approximately one-third of the value at 0°C.

Viscosity is critical in the texture development of yogurt and cultured milks. It is a crucial attribute in defining mouthfeel, flavor release, and refreshing quality of the product. It forms an important parameter in quality control programs of culture dairy plants. In yogurt, the viscosity is of the order of 15,000 to 25,000 centipoises.

64.6.9 Surface Activity

It is involved in adsorption phenomena and the formation and stability of emulsions. It is relevant to creaming, fat globule membrane function, foaming, and emulsifier use in dairy products. Normal cow milk has an inherent surface activity. Its surface

tension approximates 70% of that of water (72 dynes/cm). The surface tension of cow whole milk ranges from 50 to 52 dynes/cm, and for skim milk, 55–60 dynes/cm at 20°C. For cream it is approximately 46–47 dynes/cm. Casein, along with the proteolysis products protease-peptones is largely responsible for the surface activity. Whey proteins make little contribution. Fat reduces surface tension by a physical effect. Lactose and most of the salts tend to raise it when they are present in true solution.

Surface tension decreases as milk temperature rises. Processing treatments such as heating, sterilization, homogenization, and shear tend to increase surface tension. However, homogenization of imperfectly pasteurized milk or contamination of homogenized pasteurized milk with raw milk causes partial hydrolysis of milk fat resulting in low surface tension, bitter flavor, and rancidity of milk.

64.6.9.1 Foaming. Milk and milk products high in milk fat and/or milk proteins interact frequently with air and form foams. Sometimes the process is desirable as in whipping of cream and sometimes it has a nuisance value as in handling of skim milk. Fat globules and free fat make foam less stable. Heating of milk to such an extent that whey proteins are denatured yields more voluminous and more stable foam on heating. However, sterilization diminishes its foaming capacity. A concentrate of 30% dry matter that has been vigorously homogenized forms very stable foam.

Foaming of milk is minimum at 30–35°C. At 60°C, the foam volume is independent of the fat content. Below 20°C and above 30°C, the foaming tendency appears to increase. Fat tends to stabilize the foam formed below 20°C, for instance, during churning for butter production. Skim milk produces slightly more stable foam above 30°C than whole milk or light cream.

The formation of stable foam depends upon two main factors. First, the lowering of the surface tension allows the gathering and spreading of the surface-active components into thin films. Second, the films must be sufficiently elastic and stable to prevent the coalescence of the gas cells. Stable foam is thus formed when the surface tension of the liquid is not great enough to withdraw the film from between the gas cells and when the stabilizing agent has great internal viscosity.

Foaming properties affect handling of milk products and how dairy-based ingredients are incorporated into other products. Foam formation and its stability constitute important factors in getting the necessary overrun and texture in ice cream and frozen dairy desserts including frozen yogurt and whipped yogurt. Many yogurt plants use antifoaming agents as processing aid to control foam formation during the preparation of yogurt mix. Foam control is also necessary from proper pasteurization standpoint because organisms suspended in foam are resistant to common heat pasteurization time–temperature regime.

64.6.10 Curd Tension

This property is considered important in relation to the cheese making characteristics as well as digestibility of milk. The curd tension of milk is 28–54 g. Heat treatment of milk causes a reduction of curd tension, as does homogenization treatment. Addition of some of the salts such as the sodium citrate and sodium hexametaphosphate tend to reduce the curd tension of milk.

64.6.11 Colligative Properties

64.6.11.1 Osmotic Pressure. The number of molecules or particles, not the weight of solute, control osmotic pressure; thus 100 molecules of size 10 will have 10 times the osmotic pressure of 10 molecules of size 100. It follows that for a given weight, the smaller the molecules the higher the osmotic pressure.

Milk is formed from blood, the two being separated by a permeable membrane; hence they have the same osmotic pressure. In other words, milk is isotonic with blood. The osmotic pressure of blood is remarkably constant although the composition, as far as pigment, protein and so on, are concerned, may vary. The osmotic pressure is basically a function of salt balance and lactose content of milk.

64.6.11.2 Freezing Point. Pure water freezes at 0°C. Milk freezes at a temperature slightly lower than water due to the presence of soluble constituents such as lactose and soluble salts. Freezing point of milk depends on molar concentration of its soluble, low molecular weight compounds. Lactose, potassium, sodium, and chloride are the principal milk constituents responsible for 75–80% of the entire freezing point depression. Since it is a fairly constant property of milk, it is routinely used for detecting adulteration of incoming milk with water, using a cryoscope. Adulteration of milk with water lowers the molal concentration of lactose and salts, and thus increases the freezing point. Earlier work was done with Hortvet Cryoscope using mercury-in-glass thermometer and results on freezing point were based on Hortvet scale. More recent work with thermistor measuring devices has shown that Celsius and Hortvet scales are not identical. The following relationship has been reported (Harding 1995).

$$^{\circ}\mathrm{C} = 0.96418^{\circ}\mathrm{H} + 0.00085$$
$$^{\circ}\mathrm{H} = 1.03711^{\circ}\mathrm{C} - 0.00085$$

Accordingly, −0.540°H is actually −0.521°C. Most of the industry data is reported in °H. Freezing point depression is positive version of freezing point. Accordingly, freezing point depression of 0.540°H is equivalent to freezing point of −0.540°H. On adulteration with water, zero degree being the reference point, freezing point depression (FPD) decreases, while freezing point registers an increase. Milk from individual cows shows a narrow range in their FPD (0.530–0.525), but pooled milk has average of 0.543°H. It is generally agreed that milk of FPD higher than 0.535°H may be presumed to be water free. But readings between 0.530°H and 0.534°H warrant a letter to the supplier for a check on their plant operation. When FPD readings are between 0.525°H and 0.529°H, there is a strong suspicion of added water to the milk. Any time, the reading is 0.525°H or less, assumption of extraneous water in milk is justified.

We will now illustrate how freezing point method detects adulteration of milk with extraneous water. Milk with no added water should freeze at −0.540°C. When 10% water is added, its freezing point should be −0.486°C. As little as 3% water added to milk can be detected by this method. In general, percentage added water is calculated as follows:

$$\%\ \text{added water} = \frac{0.540 - \text{FPD}}{0.540} \times (\text{100-total milk solids})$$

Fermented milks show significant lowering in FPD because of conversion of lactose to lactic acid and transformation of minerals to ionic form. The freezing point of cream, skim milk, and whey are identical with that of the milk from which they are prepared. Therefore, the freezing point test does not detect the addition of skim milk or removal of fat from milk samples. Moreover, watered milk, which has soured, may pass the test because souring results in an increase of the FPD due to an increase in the amount of soluble molecules. Hence, the freezing point should be determined in fresh samples (having no developed acidity) for greatest accuracy.

64.6.11.3 Boiling Point. A solution boils at a higher temperature than does the pure solvent, according to the concentration of the dissolved substance. The milk constituents in true solution are mainly responsible for the elevation of the boiling point above 100°C. Elevation of the boiling point is based on the same principles as depression of freezing point. However, for detecting added water, the freezing point method is far superior on the grounds of accuracy and convenience. The boiling point of milk is 100.17°C.

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65

Genetics and Milk Production

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65.1 Introduction 380
65.2 Interspecies and Intraspecies Genetic Variation 380
65.2.1 Production 380
65.2.2 Composition 382
65.2.2.1 Milk Fat 382
65.2.2.2 Milk Sugar 385
65.2.2.3 Milk Protein 386
65.2.2.4 Milk Enzymes 387
65.2.2.5 Milk Vitamins and Minerals 388
65.2.2.6 Milk Somatic Cell Content 389
65.2.3 Implications 389
65.2.3.1 Milk Fat 389
65.2.3.2 Milk Sugar 390
65.2.3.3 Milk Protein 391
65.2.3.4 Milk Enzymes 394
65.2.3.5 Milk Vitamins and Minerals 394
65.2.3.6 Milk Somatic Cell Counts 394
65.3 Summary/Conclusion 395
References 395

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65.1 INTRODUCTION

Throughout the world, large domestic livestock such as cows, goats, sheep, water buffalo, and to lesser degree, other mammals including horses (Malacarne and others 2002), camels (Al-Awadi and Srikumar 2001), and even reindeer and African eland are utilized for their milk. Equally reputable sources report different concentrations of components for individual milk components and production capacities of various mammals. Variability in reported values is not surprising, considering that milk yield, somatic cell count and milk components are all influenced by breed, parity, type of birth, nutrition, environmental conditions, stage of lactation, and genetic variability across and within species (Gonzalo and others 1994; Goonewardene and others 1999). Interspecies variability likely results from physiological metabolic process differences in the species as well as varying needs of the respective young (Jenness 1988).

Intraspecies variability in milk production and individual milk components results from genetic mutations, parity, multiple births, season, stage of lactation, and so on. For instance, milk production is stimulated by long-day photoperiod (Dahl and others 2000). Milk production, and subsequent lactation casein yield, typically increases as parity increases (Hayes and others 1984). Similarly, milk yield increases with number of offspring in a single birth (Goonewardene and others 1999). Within a single lactation, milk production reaches a peak after about 4 weeks, remains relatively high up to 5 or 6 months, after which production decreases rapidly (Bertilsson and others 1997). However, fat and protein production are inversely related to milk production. With advancing age of cows, whey protein concentration increases, while β-casein concentration declines (Ng-Kwai-Hang and others 1987). Finally, milk production tends to decrease in times of external stress such as weather extremes or injury, and serum protein is more subject to environmental influences than casein (Hayes and others 1984). In summary, lactation is influenced both by genetic and environmental factors (Togashi and Lin 2004). This chapter will focus on several genetic factors that influence variability in milk composition within and across ruminant species, and the implications of compositional differences on dairy products.

65.2 INTERSPECIES AND INTRASPECIES GENETIC VARIATION

65.2.1 Production

In the United States, cows are the species of choice for milk volume production. The commercial significance of cow milk ensures that it is the most exhaustively studied of all milks. As of December 31 2005, 8.2 million milk cows were reported to be in the 23 largest milk-producing states. This number is up 82,000 cows from the previous year. For the year 2005, milk production in those top 23 states totaled 73.1 billion kg (National Agricultural Statistical Service 2006).

Although cows predominate in the relatively pampered conditions that define western dairy farms, elsewhere in the world, goats, sheep, and water buffalo are commonly utilized for their durability in extreme conditions. Goats are utilized in areas where grass and other feeds are sparse. The goat typically covers 10 km per day foraging and makes use of browse (Haenlein 2001), yielding an advantage in climates that experience seasonal droughts and subsequent exhaustion of grass resources. The goat also has a high digestive rate, permitting a more rapid passage of poor quality feeds (Haenlein 2001).

TABLE 65.1 Production and Composition of Cow, Goat, and Sheep Milk Compared.

	Cow	Goat	Sheep
Component (%)	Mean	Mean	Mean
Water	87.1	87	83.2
Protein	3.4	3.6	5.9
Casein	2.7	2.9	4.7
Whey	0.7	0.8	0.9
Fat	3.3	4.1	7.0
Lactose	4.8	4.5	5.3
Ash*	0.7	0.8	0.9
Production (kg/day/animal)	18–23	1–5	0.5–2
Cheddar cheese yield (%)	10%	9%	20%

Source: Adrizzo 1992; Haenlein and Ace 1984; Holsinger 1982; Jenness 1980; Loewenstein and others 1980; Morand-Fehr 1982; Ramos and Juarez 1986.

At the end of 2002, there were 1.3 million goats in the United States, and worldwide, more people consume the milk of goats than of any other species (Haenlein 2001; Anonymous 2002). In 1988, the total worldwide milk production was 524 million tons. Of that total, goats and sheep accounted for about 8.2 tons (7420 kg) and 9.0 tons (8145 kg), respectively (Anonymous 2002). In addition to goats being used as an alternative to cows for milk production, water buffalo have also been used, as they prosper in the tropical environments in which they evolved. They are especially efficient as a dual use animal in less developed Asian countries where they are also used for draft (Thu 1997), and can outperform cows (Thu 2003) when fed regional crop residues, such as rice straw (Thu 1997).

While cows produce the greatest quantity of milk per animal, cow milk typically contains more water than other species with lower production capacities (Table 65.1). Sheep milk has significantly more total solids than cow or goat milk, and is a rich source of fat and protein, particularly casein. As approximately 90% of the fat and 90% of the protein from milk are retained in cheese, it is logical that sheep milk has a higher cheese yield compared to the cheese yield of cow milk. The reason why cheese yield from goat milk is not higher than cow milk, though it would be expected to be higher than reported, will be explained later in this chapter. Mammals producing milk with even higher nutrient density than the reported species include rabbit, reindeer and whale, with differences attributed to the specialized nutrient needs of their young.

A great deal of genetic variation in milk production and milk composition can be observed for the common U.S. cow and goat breeds (Tables 65.2 and 65.3, respectively). The Holstein cow breed stands out as the milk volume producer, while Jerseys stand out as

TABLE 65.2 Influence of Breed on Gross Composition of Cow Milk.

Breed	Water (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Weight† (kg)
Jersey	85.6	5.1	4.0	4.8	0.8	5060
Guernsey	86.0	4.7	3.8	4.7	0.8	5270
Ayrshire	87.2	4.0	3.5	4.6	0.7	5760
Brown Swiss	86.9	4.0	3.66	4.9	0.7	6100
Holstein	87.8	3.5	3.3	4.7	0.7	7360

Source: Webb and others 1974.

TABLE 65.3 Breed Averages for Milk Production by Registered U.S. Dairy Goats.

Breed	Fat (%)	Protein (%)	Production (kg)
Nubian	4.5	3.7	966
Oberhasi	3.4	2.9	952
LaMancha	4.1	3.1	1000
Toggenburg	3.2	2.7	855
Alpine	3.4	2.9	1002
Saanen	3.4	2.9	1092

275–305-Day lactation.
Source: ADGA 2003.

highest milk component producers. For goats, Nubians produce milk with the richest composition, while Saanens lead in milk volume production. Although the implications of differences in production capacity are obvious and translate to differences in overall volumes of milk, the implications of component differences are worthy of greater elaboration and will be discussed individually.

65.2.2 Composition

65.2.2.1 Milk Fat. For all species, fat is the most variable component in milk. The contributions that fat makes to the flavor of dairy products has driven research into that component. In humans and cows, fat composes roughly 4% of the milk, but the proportion can rise to as high as 50% among marine mammals (Oftedal and Iverson 1995). Fat possesses a high caloric density, while exerting little osmotic pressure, and permits delivery of large quantities of energy to the suckling neonate.

What stands out most when comparing the fatty acid (FA) composition of human and ruminant milk is the predominance of short and medium chain length FAs in ruminant milk. For the food scientist, these short and medium chain FAs are particularly important as flavor elements. The presence of short and medium chain FAs is likely dictated by the requirement that the milk fat of each species possess a melting point near the body temperature of the animal. Short and medium chain FAs in ruminants substitute for the unsaturated FAs in nonruminants in depressing milk fat melting points.

Milk fat, composed primarily (97%) of triacylglycerides, is synthesized in the mammary gland, either *de novo* (new) or from blood precursors. Short chain FA (C : 4 to C : 14) components of milk fat are synthesized *de novo*, almost exclusively, in the cytoplasm of mammary gland epithelial cells (Table 65.4). In all species, *de novo* synthesis requires

TABLE 65.4 Proportional Contribution of Sources of Fatty Acids (FA) in Cow Milk.

Fatty Acid	% of FA from De Novo Synthesis	% of FA from VLDL Fatty Acid
C4–C10	100	0
C12	80–90	10–20
C14	30–40	60–70
C16	20–30	70–80
C18	0	100

Source: Iverson and Oftedal 1995.

short carbon chains (acetyl-CoA), and reducing equivalents (nicotinamide adenine dinucleotide phosphate, reduced form). In the ruminant, the carbon sources used for FA synthesis are acetate and β-hydroxybutyrate (Grummer 1991) while in nonruminants, glucose is used for a carbon source in FA synthesis (Dils 1983). Similarly, about half of the palmitic acid (C : 16) found in milk is synthesized in mammary tissue (Mansbridge and Blake 1997). This *de novo* fat synthesis in the mammary gland accounts for approximately half of the total milk fat, depending upon the species. The remaining fat is derived from the blood. Early in lactation, these fats are mobilized from endogenous stores, but as the lactation progresses, the diet becomes the largest contributor (Grummer 1991).

In nonruminants, since the FA composition of the diet can directly affect the FA composition of the milk, feeding supplemental dietary fat can increase milk fat yield and FA composition of the milk fat (Iverson and Oftedal 1995). Ruminant milk fat composition is not greatly influenced by diet, as diets are typically low in lipids and dietary lipid is metabolized by rumen microflora (Grummer 1991). One notable exception is the antitumorigenic conjugated linoleic acid (CLA), which can be increased in milk by feeding the ruminant a diet high in linoleic acid (Kelly and others 1998) CLA results from the incomplete reduction of linoleic acid by rumen microflora. FA profiles can be manipulated to a degree with bypass fats, which allow the lipids to pass directly to the intestine and become part of the FA profile of the VLDL and chlyomicrons (Jensen 2002; Perfield and others 2002; Sanz and others 2004). Bypass fats are encapsulated or otherwise protected from the rumen environment.

In addition to containing higher percentages of fat (Table 65.1), goat and sheep milks naturally contain a higher proportion of short-chain volatile FAs than cow milk (Haenlein 2001). The volatile FAs, including butyric acid (C : 4), caproic acid (C : 6), caprylic acid (C : 8) and capric acid (C : 10), have characteristic aromas and flavors, commonly referred to as "goaty." These short chain FAs could not contribute to the evolution of "goaty" flavors in milk were it not for lipolysis. Short chain FAs are preferentially esterified in the labile *sn*-3 position of the triacylglyceride, though the preference diminishes as the chain lengthens. Similarly, lipase preferentially de-esterifies the *sn*-3 position four-to-one over the *sn*-1 position (Jensen and Newberg 1995). As such, the incidence of shorter chain FAs in the *sn*-1 position contributes disproportionately to flavor development. Even refrigerated milk can undergo lipolysis due to the action of lipoprotein lipase (LPL). Although cow milk contains high levels of endogenous LPL, the rate of "spontaneous lipolysis" in cow milk is less than in goat milk, possibly due to the differing compartmentalization of LPL. In goat milk (and human milk), LPL is bound to the milk fat globule membrane. However, in cow milk, LPL is bound to the casein micelle (Chilliard and others 2003). This compartmentalization can be disrupted by handling. Both rapid cooling and rough mechanical treatment of raw milk can dislodge LPL from the casein micelle, permitting it to reattach itself to the milk fat globule, facilitating spontaneous lipolysis (Swaisgood 1995).

It is notable that goat milk contains a small amount of 4-ethyldecanoic acid, noted for imparting a distinctly "goaty" flavor or smell. This FA has the lowest flavor threshold level yet determined and may serve the goat as a pheromone (Brennand and others 1989). Additionally, individual caprine breeds exhibit a wide variability in LPL activity and its attendant rate of spontaneous lipolysis. The LPL activity measured in Norwegian goat milk is 70 μm FA lipolyzed/h/mL, which correlates to a spontaneous lipolysis rate of 4.6 mmol of FFA/day/L. In Alpine goat milk, these activities are 35 μm FA lipolyzed/h/mL, with a spontaneous lipolysis rate of 0.5 mmol of FFA/day/L. In Saanen milk,

the activities are 21 μm FA lipolyzed/h/mL and the spontaneous rate is 1.1 mmol of FFA/day/L (Chilliard and others 2003). Thus, one would expect the Norwegian goat to exhibit the most rapid spontaneous development of goaty flavors. It is important to note that the rate of spontaneous lipolysis is not directly proportional to total LPL activity.

Among commercially milked species, all exhibit a high degree of saturation (Table 65.5). Saturated FAs compose 70%, 69%, 68%, and 66% of total fat in buffalo, sheep, goats, and cows, respectively. Polyunsaturated FAs are limited in these species, with sheep milk possessing the highest degree of polyunsaturation and buffalo milk the lowest (Table 65.5). All of the unsaturated FAs have long chain lengths. Monounsaturated FAs range from 26% to 30% in all species presented.

Goat milk may be described as "naturally homogenized" because little or no surface cream layer forms during storage. This is the result of two qualities of goat milk. First, goat milk lacks agglutinins (Jenness 1980; Attaie and Richter 2000; Haenlein 2001). Agglutinins, including immunoglobulin M, are "sticky" proteins that aid in the agglomeration or clumping of fat globules in cow milk (Huppertz and others 2003). Second, goat milk contains a higher proportion of small fat globules than cow milk (Attaie and Richter 2000; Haenlein 2001). Individual goat milk fat globules range from approximately 0.7 to 8.6 μm in diameter, while cow milk fat globules range from approximately 0.9 to 15.8 μm in diameter (Attaie and Richter 2000). Although higher in fat content, ewe milk also creams slowly both at room and refrigerator temperatures (Fahmi and others 1956a). The range and size distribution of ewe milk fat globules closely resembles those of goat milk (Fahmi and others 1956b). In a comparative study, Attaie and Richter (2000) found that 90% of the fat globules in goat milk were less than 5.1 μm in diameter,

TABLE 65.5 Fatty Acid Profiles of Milk from Different Species.

	Cow	Goat	Sheep (g/100 g of milk)	Buffalo	Human
Saturated total	2.08	2.67	4.60	4.60	2.01
4:0	0.11	0.13	0.20	0.28	–
6:0	0.06	0.09	0.14	0.15	–
8:0	0.04	0.10	0.14	0.07	–
10:0	0.08	0.26	0.40	0.14	0.06
12:0	0.09	0.12	0.24	0.17	0.26
14:0	0.34	0.32	0.66	0.70	0.32
16:0	0.88	0.91	1.62	2.00	0.92
18:0	0.4	0.44	0.90	0.68	0.29
Monounsaturated total	0.96 g	1.11	1.72	1.79	1.66
16:1	0.08 g	0.08	0.13	0.14	0.13
18:1	0.84	0.98	1.56	1.57	1.48
20:1	Trace	–	–	–	0.04
22:1	Trace	–	–	–	Trace
Polyunsaturated total	0.12	0.15	0.31	0.15	0.50
18:2	0.08	0.11	0.18	0.07	0.37
18:3	0.05	0.04	0.13	0.08	0.05
18:4	Trace	–	–	–	–
20:4	Trace	–	–	–	0.03
20:5	Trace	–	–	–	Trace
22:5	Trace	–	–	–	Trace
22:6	Trace	–	–	–	Trace

Source: USDA 2004.

while 90% of the fat globules in bovine milk were less than 6.4 μm. Camel (*Camelus dromedarius*) milk also exhibits a very slow creaming rate for both raw and heated milk at refrigerator and room temperatures, even though fat globule size distribution is similar to cow milk (Farah and Ruegg 1991). It was concluded that insufficient quantity of agglutinin in camel milk was mainly responsible for the slow rate of creaming (Farah and Ruegg 1991). Supporting data can be found comparing creaming rates of cow and buffalo milks. Although the volume of the milk fat globule of the water buffalo is double that of the cow, its creaming rate resides between milks of goats and sheep and cows (Fahmi and others 1956a). Were globule size the sole contributing factor, buffalo milk would cream faster than cow milk.

65.2.2.2 Milk Sugar. In contrast to fat, lactose is not highly variable within commercially utilized species or individual animals. This lack of variability is dictated by the physiological requirement that milk and blood possess the same osmolarity. The health of the lactating animal requires that milk and blood must be in osmotic balance, though not necessarily in equilibrium. While proteins and minerals contribute to the osmolarity of milk, the primary osmole is milk sugar. In animals of commercial importance, this sugar is lactose. As the primary milk osmole, milk production (in terms of volume) is largely dictated by lactose synthesis (Kuhn 1983). Although milk production is highly correlated with glucose uptake by the mammary gland, blood glucose availability is not generally a limiting factor in cows (Miller and others 1991). This is critical, as essentially all glucose available to a ruminant is derived from gluconeogenesis since little glucose survives the rumen for absorption in the small intestine.

Lactose is synthesized in the Golgi lumina. The Golgi membrane is impermeable to lactose, so water must be drawn into the lumen to restore osmotic balance. This results in an observable swelling of the Golgi preceding its exocytotic attachment to the apical membrane. There may be more water absorption across the apical membrane, but ultimately the milk will achieve an osmotic pressure of 6.6 atmospheres, the same as blood (Mepham 1987).

Lactose, a disaccharide composed of glucose and galactose, is only found naturally in milk serum and it is structurally identical across all species. As such, goat milk must not be used as a substitute for cow milk for lactose intolerant patients. Among species, lactose ranges from absent (marsupials and polar bears), to as high as 5.6% in human milk (Mepham 1987). In humans, the osmotic consequence of this high lactose content is offset by exceptionally low protein, especially casein, and a low mineral content (Davies and others 1983).

In marsupials, lactose is replaced by the trisaccharide *O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-*galactopyranosyl-(1 → 4)*-D-glucose (3′-galactosyl-glucose) (Messer and others 1980). In polar bears, an entire spectrum of polysaccharides fulfill the role of lactose (Urashima and others 2000). Oligosaccharides also make a considerable contribution to the carbohydrate content of human milk. These oligosaccharides compose up to 1.4% of the content of human milk (Coppa and others 1999), exceeding protein as the third greatest component of milk. Some of these oligosaccharides are also found in ruminant milk, although at a much lower concentration (Martin and Addeo 1996). While lactose is critically important in fermented dairy products and must be managed in frozen dairy products, the significance of these oligosaccharides to dairy product manufacture is even less well understood than its role in neonatal nutrition. The evolutionary purpose of these oligosaccharides has not been clearly elucidated, although some

oligosaccharides seem to possess antibacterial and antiviral activities (Kawakami 1979; Dai and others 2000) in the human intestine and may defend against gastrointestinal infections by favoring bifidobacterial colonization of the infant intestine (Rueda and others 1998; Newburg 2000). As such, it seems conceivable that these oligosaccharides might also influence microbial growth in dairy products.

65.2.2.3 Milk Protein. The milk of cows, goats, and sheep all contain the major caseins, alpha$_{s1}$-(α_{s1}-CN), alpha$_{s2}$-(α_{s2}-CN), beta-(β–CN), and kappa-(κ–CN) and whey proteins, beta-lactoglobulin (β–LG) and alpha-lactalbumin (α–LA) (Bouniol and others 1989; Brignon and others 1990; Bouniol and others 1993). However, the quantities and amino acid structure of each protein vary among and within species (Ono and Creamer 1986). The structure of cow and goat milk casein micelles, as well as the four proteins that make up the micelles, are remarkably similar (Brignon and others 1990), however the proportion of the casein and the size of the micelles differ. Average micelle diameter is 175, 255, and 202 nm in the milk of cows, goats, and sheep, respectively (Brulé and others 2000). Casein composition in caprine and bovine milks is summarized in Table 65.6. While the predominant casein in cow milk is α_{s1}-CN, β-CN predominates in goat milk. This has implications for cheesemaking, which will be discussed in more detail later in this chapter.

In cow milk, submicellar aggregates are primarily assembled from α_{s1}-CN : κ-CN and α_{s1}-CN : β-CN, which are held together with hydrophobic bonds and salt bridges (Ono and Creamer 1986; Walstra 1990). However, in goat milk, β-CN self-association complexes and κ-CN : α_{s2}-CN complexes prevail because α_{s1}-CN is limited (Brulé and others 2000; Ono and Creamer 1986). κ-CN appears to have a similar size-determining role and surface function in both species' micelles (Ono and Creamer 1986; Walstra 1990; Tziboula and Horne 1996).

Goat milk micelles have greater mineralization, calcium, and phosphate, and lower hydration than cow milk micelles, possibly accounting for the lower heat stability and more ready sedimentation and coagulation of goat micelles even at cool temperatures (Jenness 1973; Ono and Creamer 1986; Brulé and others 2000). At low temperatures, β-CN dissociates from the micelle as seen in cow milk, but more freely (Ono and Creamer 1986).

Genetic variants in the amino acid sequence of cow milk proteins have been well documented (Eigel and others 1984; Gonyon and others 1987; Huang and others 1994). Gonyon and colleagues (Gonyon and others 1987) suggested that genetic differences account for approximately 25% of the variation in milk yield and as much as 50% of the variation for fat, protein and solids-non-fat in milk. Genetic variants not only influence milk composition, but also gelation properties and cheese-producing ability (Aleandri and others 1990; Huang and others 1994). For instance, Aleandri and others (1990) showed

TABLE 65.6 Average Casein Composition of Caprine and Bovine Milks.

Casein	Percentage in Caprine	Percentage in Bovine
α_{s1}-CN	4–26	36–40
α_{s2}-CN	5–19	9–11
β-CN	42–64	34–41
κ-CN	10–24	10–13

Source: Walstra and Jenness 1984.

that α_{s1}-casein genotypes significantly influenced milk yield, fat yield, and protein yield. Bovenhuis and others (1992) reported that the β-LG gene or a very closely linked gene affects fat percentage, while κ-CN or a very closely linked gene affects protein percentage in milk. They reported that effects of β-CN genotypes on milk production, fat percentage, and protein yield were significant. The β-LG genotypes also had important consequences on milk production and protein yield, but the authors could not conclude whether those outcomes were due to effects of milk protein genes themselves or to effects of linked genes (Bovenhuis and others 1992).

Although percent protein in cow milk is positively influenced by α_{s1}-CN and κ-CN genotypes, an increase in percent protein does not necessarily increase lactation protein yield because total milk yields may actually be lower (Aleandri and others 1990). A balance must be achieved between milk yield and the ability of the milk to be made into cheese, if cheese is the goal. Aleandri and others (1990) looked at associations between casein and whey protein genotypes in cow milk and found that some protein combinations result in better cheesemaking ability than others. It was concluded that selection can and should be based on genetic combinations that will lead to optimal cheese production, rather than on the basis of protein or fat content of the milk (Aleandri and others 1990).

The primary structures of the four caseins have been reported for goat milk (Richardson and Creamer 1973; Boulanger and others 1984; Bouniol and others 1989; Brignon and others 1990). Caprine α_{s1}-CN has 88% and 97% sequence homology with its bovine and ovine counterparts, respectively (Brignon and others 1989). α_{s1}-CN is particularly interesting because it influences milk, fat and protein yields and rennet coagulation properties of cow and goat milk (Ambrosoli and others 1988; Mariani and others 1988; Aleandri and others 1990). Goat milks with high α_{s1}-CN tend to have a greater content of total solids (including fat, protein, casein, and phosphorus), lower pH, and higher curd firmness and cheese yield than milks with low α_{s1}-CN (Ambrosoli and others 1988; Martin and Addeo 1996). Goat milks with low levels of α_{s1}-CN tend to exhibit shorter coagulation time and weaker resistance to heat treatments than milks with high levels of α_{s1}-CN (Ambrosoli and others 1988; Mora-Gutierrez and others 1993).

Genetic variation has been reported for goat α_{s1}-CN, α_{s2}-CN, β-CN, and κ-CN (Richardson and Creamer 1974; Brignon and others 1990; Martin and Addeo 1996). Genetic variation of goat α_{s1}-CN alone is under the control of approximately 10 alleles, which differ by substitutions or deletions of amino acids in the genetic backbone of each protein (Grosclaude and others 1987; Mahe and Grosclaude 1989; Brignon and others 1990; Martin and Addeo 1996). Grosclaude and colleagues (Grosclaude and others 1987) observed a positive correlation between polymorphisms of α_{s1}-CN and the total casein content in milk and suggested that each genetic variant plays a role in dictating α_{s1}-CN synthesis. Casein content was found to be higher in milks containing the "high type" alleles (α_{s1}-CNA, B, and C), and lower in milks containing "low type" alleles (α_{s1}-CND, F, and G) (Grosclaude and others 1987; Brignon and others 1990; Martin and Addeo 1996). The relative amounts of α_{s2}-CN, β-CN, and κ-CN increase as the relative amount of α_{s1}-CN decreases (Grosclaude and others 1987; Law and Tziboula 1992).

Research on protein polymorphism in sheep milk is limited. Apparently α_{s1}-CN and β-LG are polymorphic, but not in every breed (Brulé and others 2000). More research should be conducted in this area to confirm genetic variability and wider implications.

65.2.2.4 Milk Enzymes. Raw milk naturally contains low levels of endogenous enzymes, including alkaline phosphatase, plasmin, and lipase (Whitney 1988). The

absence of alkaline phosphatase after pasteurization indicates effective pasteurization was accomplished. Plasmin, which is stable at pasteurization temperature, hydrolyzes both β- and α_{s1}-CN in milk and in cheese during maturation (Banks and Williams 2004). Goat milk reportedly contains less alkaline phosphatase, lipase and xanthine oxidase than cow milk (Haenlein 2001).

Of particular interest in the category of naturally-occurring milk enzymes are lipases. It is reported that milk contains a major lipase with pH optimum 8.5–9.0, and two other lipases with pH optimum at 6.5–7.0, and at 7.9 (Jandal 1995). Lipases are associated with fat metabolism and with quality deterioration in dairy products (Jandal 1995). The lipoprotein lipase (LPL) found in milk is identical to the LPL in blood and represents a spillover from the mammary tissues (Weihrauch 1988).

Other enzymes of processing significance include lysozyme and lactoperoxidase. Lysozyme attacks the glycosidic bonds found in Gram-positive bacterial cell walls. The enzyme lactoperoxidase is employed in developing countries to preserve raw milk in the absence of refrigeration. When supplied with hydrogen peroxide and thiocyanate, lactoperoxidase will catalyze the formation of anti-Gram-positive bacteriocides (Jensen 1995a).

65.2.2.5 Milk Vitamins and Minerals. Since the purpose of milk is to serve the nutritional needs of the suckling neonate, it is expected that milk should supply micronutrient needs as well as requirements for protein and energy. As such, milk is well supplied with an appropriate balance of vitamins (Jensen 1995b) and minerals. Some of these nutrients are of importance in dairy processing, such as calcium and phosphorous, while others are primarily of nutritional interest.

As with lactose, low molecular weight water-soluble components such as minerals contribute to the osmotic balance between blood and milk. And, as noted above in the discussion of milk lactose, their total contribution to the osmotic pressure of milk must equal 6.6 atm. But milk is far richer in soluble carbohydrates, calcium, phosphorous, and potassium than blood, as these are compartmentalized in concentrations that far exceed that found in extracellular fluids. Milk contains 1/7 as much Na, five times as much K, 13 times as much Ca, and 10 times as much P as blood plasma (Peaker 1978). The chlorides and lactose in milk account for about 75% of milk's osmotic pressure.

Calcium and casein concentrations correlate closely with each other, as calcium and phosphate, and citrate, are all integral to the formation of the casein micelle in the Golgi apparatus (Neville and others 1983). Calcium is actively transported into the Golgi while phosphate appears in the Golgi as a consequence of lactose synthesis (Atkinson and others 1995). By assembling the casein complex from these building blocks, calcium and phosphorous are balanced with protein in concentrations adequate to support the rapid growth of the neonate. The cosecretion of calcium, phosphate, and casein ensures that this balance is maintained and explains why human milk is simultaneously low in calcium, protein, and phosphorous, relative to cows, goats, and sheep, and why sheep milk is highest among this group for these components.

Once again, vitamins appear in milk for use by the suckling neonate. As Darwinian fitness is determined by how successfully an individual produces viable offspring, the milk is well supplied with vitamins. That water-soluble vitamins appear in milk as a result of active transport is evidenced by the fact that vitamin levels are maintained in the milk even during episodes of maternal depletion (Picciano 1995).

65.2.2.6 Milk Somatic Cell Content. Somatic cells, including lymphocytes, neutrophils, and epithelial cells, are naturally higher in goat and sheep milks than in cow milk (Leitner and others 2004). While high somatic cell counts (SCC) are associated with mastitis in cow (Politis and Ng-Kwai-Hang 1988) and sheep milk (Gonzalo and others 2002), goats may have high SCC, even in the absence of mammary infection (Droke and others 1993; Haenlein 2002). Nonleucocytic cell-like particles are normally observed in goat milk as a result of the apocrine secretion process of the goat mammary gland (Oliszewski and others 2002). The cytoplasm of the milk-making cell (alveolus) breaks off when goat milk is produced in the udder (apocrine process), while little cell destruction occurs during production of cow and sheep milk (merocrine process). Because most somatic cell enumeration techniques do not distinguish between different cells, testing equipment and procedures are of limited reliability and applicability to goat milk, unless appropriate correction factors and calibration with goat milk are used. The only reliable method for arbitration seems to be the pyronin Y-methyl green stain (Haenlein 2002). The pyronin Y-methyl green stain test confirmed that bulk tank milk from commercial dairy goat herds in California, Arkansas, Michigan, and Wisconsin contained 9.9% macrophages, 2.8% lymphocytes, and 87% neutrophils (Droke and others 1993). SCCs tended to be high (greater than 1,000,000 cells/mL) and increased neutrophils contributed to high SCC in the bulk tank goat milk (Droke and others 1993). These species differences are reflected in the disparate standards for SCCs in cow and goat milk. Grade A goat milk is allowed a higher legal limit of somatic cells (1,000,000 cells/mL) than cow milk (750,000 cells/mL) (U.S. Department of Health and Human Services 1999).

65.2.3 Implications

65.2.3.1 Milk Fat. Since casein and fat are the principal components of cheese, milk fat content will obviously have implications upon cheese yield. In fact, the ratio of casein to fat is often standardized in cheesemaking, in order to ensure consistency and optimize yield. Failure to standardize can result in reductions in yield efficiency, lack of moisture control during manufacture, and production of substandard cheese that does not meet specifications for fat in dry matter (Banks and Williams 2004). From a nutritional standpoint, the amount of fat in a given cheese varies more in response to cheese making practices and moisture content than by genetic and species differences.

Due to the vulnerable nature of milk fat globule membranes, the presence of LPL, and the presence of unsaturated fats, milk fat of all species is vulnerable to a number of quality defects. Raw milk must be handled especially carefully, as LPL is active until pasteurization. Raw milk must not be excessively agitated or allowed to foam because milk fat globule membranes can be disrupted, enabling LPL attack on triacylglycerols. Raw milk must never be mixed with pasteurized milk, as the active LPL can enter the sensitized milk fat globules damaged by, and reformed after homogenization, causing lipolysis. Goat and sheep milk, with their greater structural exposure to LPL and higher concentration of short chain FAs in the *sn*-3 position, appear to be especially prone to rancid off-flavors.

It should be noted, however, that rancid flavors are often appreciated in cheeses, particularly feta, Romano, Roquefort, and blue. Goat milk cheeses naturally have piquant, pepper-sharp flavors, while sheep milk cheeses are smooth, mellow, and highly aromatic (Kosikowski and Mistry 1997). The high fat content of sheep milk does not necessarily translate to high fat content in cheeses (Table 65.7).

TABLE 65.7 Typical Compositions of Sheep, Cow, and Goat Hard Cheeses.

	Sheep Cheese Varieties			Cow Cheese Varieties			Goat Cheese Varieties	
Component	Feta	Manchego	Roquefort	Cheddar	Gouda	Blue	Metsovone	Corfu
Water (%)	47	43	39	38	43	42	42	36
Fat (%)	25	27	32	32	30	30	26	27
Protein (%)	19	25	21	25	25	20	27	30
Salt (%)	4	2	4	2	2	5	3	4
FDB[a] (%)	47	47	52	52	53	52	45	42

[a]Fat on a dry basis.
Source: Anifitakis 1991; USDA 1978; Kosikowski and Mistry 1997.

Although pasteurization inactivates LPL, unsaturated FAs remain susceptible to the attack of light, metals, and free radicals (auto-oxidation). To minimize the potential for development of metallic off-flavors, milk should not contact copper or iron pipes or fittings (Bodyfelt and others 1988). Light-induced oxidation always occurs in glass and plastic consumer packages not shielded with UV-barriers. The extent of auto-oxidation is time dependent. Minor differences in levels of oxidation can be noted only by individuals trained in the art of detection, so minor differences in level of unsaturation in FAs among species, breeds, or animals are not of practical significance with respect to oxidation.

Goat enthusiasts frequently tout the digestibility of goat milk compared to cow milk, so a report on supportive research is appropriate for this chapter. Several investigators have studied the digestibility of goat milk compared to cow milk (Alfreez and others 2001; Barrionuevo 2002). The digestive utilization of fat was greater in rats receiving a diet of goat milk (rich in medium-chain triglycerides) than those given a cow-milk-based diet, and more closely approached the digestibility values obtained for olive oil. The consumption of goat milk reduced levels of cholesterol in the rats while levels of triacylglycerides and high-density lipoprotein remained within the normal ranges for the rats (Alfreez and others 2001). In a similar study, the apparent digestibility coefficient for iron in rats was highest with the goat milk diet, followed by the standard diet and lowest with the cow milk diet (Barrionuevo and others 2002). The apparent digestibility coefficient of copper was higher in the animals fed the goat milk diet than that in the two groups of animals fed the other diets. The studies suggest a beneficial effect of goat milk, with respect to cow milk, on the metabolism of fat, iron, and copper in control rats, especially those with malabsorption syndrome (Alfreez and others 2001; Barrionuevo and others 2002).

65.2.3.2 Milk Sugar. The presence of lactose, rather than glucose or maltose, as the primary carbohydrate in milk is of considerable consequence in the dairy industry. The capacity to hydrolyze galactose $\beta(1-4)$ glucose is rare among microorganisms (Newburg and Neubauer 1995) and facilitates our manufacture of fermented milk products. If more bacteria were able to rapidly use lactose, our manufacture of fermented milk products would be greatly complicated. Milk from our economically important species contains a surplus of lactose for lactic acid bacteria to utilize as their primary energy source in the conversion to lactic acid in fermented dairy products. Since lactose is not limiting to microorganisms, differences in lactose content in milk of species commonly used for fermented dairy products has little practical significance.

Lactose in milk is nearly unique in nature and its nutritional consequences are not well elaborated. But one clear advantage to lactose, compared to glucose, is that in secreting a dissacharide instead of a monosaccharide, the mammary gland can deliver twice the carbohydrate to the neonate without doubling the volume of milk, as osmotic considerations would otherwise demand. Also, as mentioned above, the galactose β(1−4) bond is rare in nature and few microorganisms can metabolize it. Finally, the galactose moiety may support galactolipid synthesis and consequently, neonatal brain growth (Newburg and Neubauer 1995).

Because the chemical structure of lactose is identical among species, individuals with lactose intolerance or who are lactose maldigesters will suffer similar consequences after drinking either cow or goat milk. Lactose maldigesters either lack or have reduced production of the enzyme β-galactosidase (lactase), required for the hydrolysis of lactose into its glucose and galactose subunits for subsequent absorption. Lactose maldigesters' intestinal discomfort arises from hindgut fermentation of the lactose by intestinal microflora. Lactose maldigesters need not and should not eliminate dairy products from their diet (Miller and others 2000). A patient's tolerance to dairy foods may be influenced by the amount of lactose, type of dairy food, whether the food has been fermented or hydrolyzed with an enzyme preparation, whether the lactose-containing food is eaten with a meal, and colonic adaptation (Miller and others 2000). A concentrated form of lactase may be consumed by patients, in the form of a tablet prior to ingestion of milk or ice cream, to ease potential symptoms. Lactase is also utilized by manufacturers to make a variety of commercial products for lactose maldigesters (Lactaid® milk and ice cream). Because lactose is reduced nearly to zero in most hard cheeses, lactose maldigesters need not avoid those foods. Additionally, buttermilk, yogurt and acidophilus milk are commonly comfortably consumed by lactose maldigesters because the microbial β-galactosidase aids in digestion of lactose (Miller and others 2000).

65.2.3.3 Milk Protein. From a technological standpoint, genetic variation in proteins can have profound effects on manufacturing properties of milk, including gelation properties, cheese curd formation, and cheese yield (Marziali and others 1986c; Ng-Kwai-Hang and others 1987). Huang and others (1994) demonstrated that subtle changes, involving as few as two amino acid residues in the primary structure of β-LG, resulted in significant changes in gelation temperature and rheological properties of the gel formed. Aleandri and others (1990) reported that α_{s1}-CN genotype significantly influenced milk yield, fat yield, protein yield, and lactation cheese yield, with the highest yields obtained for genotype BB. They also concluded that cheese yield was related to β-LG genotype, with highest estimates for the BB genotype (Aleandri and others 1990). κ-CN genotype BB was associated with the highest percentage of protein in the milk (Aleandri and others 1990). Marziali and Ng-Kwai-Hang (Marziali and Ng-Kwai-Hang 1986a,c) went as far as to report that milk containing β-CN A1A1, κ-CN BB and β-LG BB would maximize the solids entering the cheese mass, thus maximizing yield. Ojala and colleagues (1997) used a more conservative, multitrait animal model to estimate effects of κ-CN, β-CN, and α_{s1}-CN on fat percentage and milk and protein production in Holsteins. A joint effect of alleles had a positive relationship with production data. The authors cautioned that before their reported composite κ-β-α_{s1}-CN genotypes or the corresponding haplotypes be used for selection to improve milk production traits, the results be confirmed using sufficiently large data for more than on breed and/or within large herds (Ojala and others 1997).

It was stated earlier that goat milk contains significantly less α_{s1}-CN than cow milk. The consequences of this feature are two-fold, since α_{s1}-CN is a structural component of the casein micelle and plays a functional role in cheese curd formation (Walstra and Jenness 1984). Low levels of α_{s1}-CN result in soft curds and soft curds are associated with low cheese yields, even when total casein levels are similar (Marziali and others 1986c; Remeuf and Lenoir 1986; Ambrosoli and others 1988; Clark and Sherbon 2000a). Clark and Sherbon (2000a,b) reported that goat milk that lacked α_{s1}-CN had lower percentages of milk components and poorer coagulation properties than milk that contained α_{s1}-CN, suggesting that the presence of α_{s1}-CN in milk should improve coagulation properties. Since percent total solids, SNF and protein were more highly correlated with coagulation properties than α_{s1}-CN itself, quantification of the former was recommended to be more practical in predicting cheese-making potential than measuring α_{s1}-CN, which is more tedious and costly. Othmane and others (2002), who studied dairy sheep, also discouraged casein content as a selection criterion in dairy sheep. However, since heritabilities for protein and casein contents were very similar in sheep, the authors concluded that protein content is an adequate, more economical measure for selection.

One consequence of genetic polymorphism in proteins is the differing amino acid composition in individual caseins and whey proteins (Ng-Kwai-Hang and others 1987), but this difference is of minor nutritional significance. A quick comparison of the nutrient profiles of the most commonly milked species with human milk (Table 65.8) reveals a considerable disparity. Most notable are the much lower protein and mineral contents of human milk. The protein difference would appear to be even more considerable when one considers that, unlike ruminant milk, serum proteins predominate in human milk, with a consequently lower casein content (Jenness and Sloan 1970).

On the other hand, the amino acid profiles of the most commonly milked species do not depart radically from the profile of human milk (Fig. 65.1), especially among the essential amino acids, making the difference one of quantity rather than quality. Even so, infant formula manufacturers supplement their products made from cow milk to more closely resemble human milk.

One consequence of consuming nonhuman milk is the increased likelihood of adverse reactions to milk components. Infants, in particular, occasionally experience either cow milk protein allergy (CMPA) or cow milk protein intolerance, depending upon whether the reaction is immunological in origin (Host 1994). The incidence of adverse reaction

TABLE 65.8 Proximate Analysis of Milk from Different Species.

Proximate Analysis	Cow milk	Goat Milk	Sheep Milk (Amount/100 g)	Buffalo Milk	Human Milk
Water g	87.99	87.03	80.70	83.39	87.5
Calories kcal	61	69	108	97	70
Food energy kJ	257	288	451	404	291
Protein N × 6.38 g	3.29	3.56	5.98	3.75	1.03
Total lipid (fat) g	3.34	4.14	7.00	6.89	4.38
CH_2O g	4.66	4.45	5.36	5.18	6.89
Fiber g	0	0	0	0	0
Ash[a]	0.72	0.82	0.96	0.79	0.2

[a]Noncombustible material.
Source: USDA 2004.

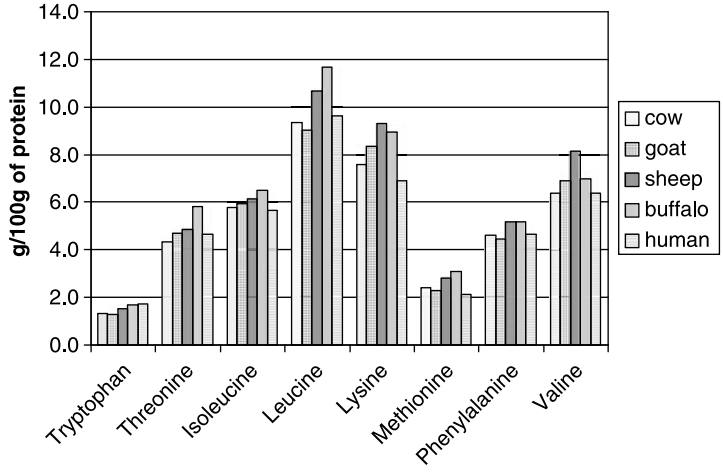

Figure 65.1 *Essential amino acid profiles of milk from various species (USDA 2004).*

to cow milk may be as high as 2% or 3%, with allergic reactions accounting for about 1.4%. Allergies may develop to any of the proteins in milk, including caseins, β-LG and α-LA (Bouniol and others 1993).

Contradictory results have been reported on the use of goat milk as an alternative to in cow milk in cases of allergy. Because of goat and cow milk composition similarities, immunologic cross-reactivity exists between many goat and cow milk proteins (Darnton-Hill and others 1987). Should a child exhibit allergic symptoms to cow milk, goat milk is unlikely to serve as a safe substitute, as there is a high rate of cross-allergenicity with cow milk (Dean and others 1993). However, due to genetic polymorphisms, cross-reactivity with goat milk proteins may not be experienced. A study was designed, using a guinea pig model of cow milk allergy, to test if discrepancies in allergic responses could be due to the high genetic polymorphism of goat milk proteins (Bevilacqua and others 2001). Fourty guinea pigs were fed over a 20-day period with pelleted diets containing either soybean proteins (group S), cow milk proteins (group CM), goat milk proteins with high (group GM1) or low (group GM2) α_{s1}-casein content. Parenteral sensitization to GM1 and GM2 proteins was measured (ADGA 2003) by systemic IgG1 antibodies directed against bovine or caprine β-LG, α-LA, and whole caseins, and (Al-Awadi and Srikumar 2001) by intestinal anaphylaxis. Guinea pigs fed on CM and GM1 developed high titers of anti-β-LG IgG1, with an important cross reactivity between goat and cow β-LG. However, in guinea pigs fed on GM2, anti-goat β-LG IgG1 antibodies were significantly decreased compared with GM1 guinea pigs, and the intestinal anaphylaxis was significantly decreased compared with that observed in GM1 guinea pigs. Animals receiving GM1 or GM2 proteins via the parenteral route developed a marked sensitization. These results suggest that discrepancies observed in the use of goat milk in cow milk allergy could be due, at least in part, to the high genetic polymorphism of goat milk proteins (Bevilacqua and others 2001).

Soy milk is a proposed alternative to cow milk formulas, but soy milk is not without its perils. In a study that switched young children with CMPA to soy milk, it was found that 14% developed allergic symptoms to the soy (Zeiger and others 1999). In addition to

allergic reactions, soy injects the added complications of feminizing phytohormones. Soy is rich in isoflavones and recent research into the effect of these compounds on humans, especially males, has given alarm (Setchell and others 1997). Infants receiving soy-based infant formulas are exposed to between six and 11 times the isoflavone dosage demonstrated to exert hormonal effects upon adults (Setchell and others 1997). Others have insisted that evidence yet revealed does not justify those concerns (Merritt and Hanks 2004).

65.2.3.4 Milk Enzymes. Raw milk is somewhat resistant to LPL because of the protective nature of the milk fat globule membrane (Weihrauch 1988). However, during milk processing (cooling, agitation, homogenization, and heat treatments), the milk fat globule membrane is altered, making it more susceptible to the action of LPL (Gervilla and others 2001). If activated by severe agitation, temperature fluctuations, or other means, LPL hydrolyzes FAs from triacylglycerides, leading to rancid off-flavors in milk and subsequent cheese. While rancid flavors may be expected and even desired in some cheeses (blue, Romano, feta), rancid milk is always offensive. Rancidity in milk may be detectable at acid degree values exceeding 1.2 meq/L (Bodyfelt and others 1988; Weihrauch 1988). Jandal (1995) demonstrated that LPL activity in both goat and cow milk increased with agitation and alkaline pH, and was reduced by pasteurization, cooling, sodium chloride addition, and acidic pH. As described previously, goat milk's reputation for being more susceptible to off flavors than cow milk is more likely due to vulnerable structure of the milk fat globule membranes (Chilliard and others 2003) than total LPL activity. Heating of milk to 80°C for 20 s. destroys all lipases in all milk (Weihrauch 1988). Regardless of species, to prevent enzymatic degradation, it is of critical importance to avoid agitation and foaming of dairy products, particularly raw milk. Pasteurization of milk soon after collection is a safeguard against rancid off-flavors in milk and subsequent manufactured dairy products.

65.2.3.5 Milk Vitamins and Minerals. As calcium and phosphorous are structural components of the casein micelles, content of these minerals in the milk will influence the end products of milk processing. Indeed, calcium is occasionally added to milk to increase curd firmness during Cheddar cheese manufacture (Lenoir and others 2000b). Calcium content is also known to influence the rheological properties of soft cheeses as well (Solorza and Bell 1998). Higher calcium content enhances rennet activity and protein–protein interactions by masking negative charges (Green and Marshall 1977).

One might then predict that the higher calcium content of sheep milk would yield a firmer cheese than cow milk. But as mentioned previously, the protein quantity and quality varies dramatically among species, making it impracticable to tease out the effects of endogenous minerals on product characteristics.

Residual calcium in the final cheese has been implicated as a contributing factor in calcium lactate crystal formation in Cheddar cheese (Dybing and others 1988).

Water soluble vitamins are satisfactorily heat stable, survive pasteurization, and find their way into most final products (Jensen 1995b). The exception is cheese, where most of the water soluble vitamins are lost in the whey because of their dissolution from the casein micelle at low pH (USDA 2004).

65.2.3.6 Milk Somatic Cell Counts. In cows, high SCCs are associated with mammary infection. SCCs of cow milk are also associated with milk coagulating

properties, including increased rennet clotting time and decreased curd firmness (Politis and Ng-Kwai-Hang 1988). Since firm curds improve efficiency of cheesemaking and reduce losses of milk components in the whey, reduced curd firmness translates to losses in cheese yield (Politis and Ng-Kwai-Hang 1988).

Regardless of SCC, mammary infections change milk composition, increase casein degradation, depress milk yield and cheese yield, and increase milk-clotting time (Leitner and others 2004). In sheep, analysis of 4352 monthly test-day records for milk yield, SCC, and bacteriology showed that uninfected ewes or ewes infected by minor pathogens had the lowest SCC and the highest milk yields, whereas those infected by major pathogens had high SCC and milk yield losses between 8.8 and 10.1% (Gonzalo and others 2002). Othmane and colleagues (Othmane 2002) demonstrated that a high SCC was accompanied by an in crease in serum protein content and involved a loss in milk yield in dairy sheep. Leitner and colleagues (Leitner and others 2004) reported a 30% reduction in milk yield in infected glands of goats, compared to the 53% reduction in milk yield of infected glands of sheep reported by Gonzalo and others (1994), suggesting greater vulnerability of sheep to subclinical mammary infections than goats. Because of the negative correlation between milk yield and SCC, El-Saied and others (1998) concluded that selection for increased milk yield alone should result in a decrease in SCC in dairy sheep.

65.3 SUMMARY/CONCLUSION

Considering that milk evolved from neonate nutrition, and was not originally intended to serve as a raw material for the food processor to make cheese, ice cream or yogurt from, nor for adolescents or adults to use as beverage, it is remarkable that milk has fulfilled these roles. The consistency, yet great variability of milks provided by different species or breeds within a species has provided us with a bountiful assortment of dairy products from which to choose. Cheeses made from sheep or goat milk possess flavors and textures not conveniently, or faithfully reproducible from cow milk. Additionally, the components of milk lend themselves to convenient modification for our palates. Understanding and appreciation of the commonalities and differences among the milks of domesticated animals, combined with technological advances in food science enables food scientists to explore and create beyond what was ever imagined when these treasured animals were first domesticated.

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66

Flavored Milks

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66.1 Introduction 404
66.2 Processing of Flavored Milks 404
66.2.1 Aspects to be Taken in Consideration for Formulating Flavored Milk 404
66.2.2 Different Flavored Milks 404
66.2.2.1 Chocolate Milk 404
66.2.2.2 Natural Orange Juice Flavored Milk 405
66.2.2.3 Banana Milk 405
66.2.2.4 Mango Flavored Milk 405
66.2.2.5 Date Milk 405
66.3 Flavored Milk With Additives 405
66.4 Flavored Milk Based Beverages 406
66.5 Flavored Carbonated Milk Beverages 406
66.6 Flavored Milk Powders 406
66.6.1 Manufacture of Powdered Flavored Milks 406
66.6.1.1 Mango Milk Powder 406
66.6.1.2 Banana Milk Shake Powder 407
66.7 Low Carbohydrate Flavored Milks 407
66.8 Flavored Milks and Nutrition 407
66.9 Myths About Flavored Milks 407
66.10 Packaging Flavored Milks 408
66.11 Future of Flavored Milks 409
References 409

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

66.1 INTRODUCTION

Flavoring milk is changing the image of milk. Flavored milks offer choices to those consumers who are not interested in plain milk. Flavored milks are milks to which a flavor along with a sweetener are added. Flavoring milk is a way of adding value to milk.

The concept of incorporating fruit flavors in milk on a commercial basis originated in 1921 when such a beverage was prepared and sold at a Wisconsin state fair. In the mid-1930s flavored milks acquired a nutritional significance of added vitamin C when oranges and lemons were blended with milk for immediate consumption (Henderson 1971). Popularity and consumption of flavored milks has increased ever since.

Incorporation of flavors makes milk more appealing to both kids and adults. Milk is flavored using various flavors such as fruits (strawberry, orange, banana, raspberry, grape), cocoa/chocolate, vanilla, coffee, caramel, cappuccino, peanut butter, root beer, and vegetable. There has been an increase in milk and nutrient intake by offering flavored milks as part of the National School Lunch Program and School Breakfast Program (Guthrie 1977, Johnson et al., 2002, Burrington 2002).

66.2 PROCESSING OF FLAVORED MILKS

66.2.1 Aspects to be Taken in Consideration for Formulating Flavored Milk

Fluid milk is about 88% water (Henderson 1971). Most flavors are water soluble so the flavors mostly stay in solution. Fruit flavors can sometimes be a bit difficult because citric acid used in these flavors can denature milk proteins. Also, at the pH of milk the citrus flavors such as lemon and orange do not taste the same.

Chocolate milks need a stabilizer system to suspend the cocoa particles so they do not settle out over time. To stabilize/suspend the cocoa particles typically kappa II carrageenan is used at a level of 0.02–0.03%. Carrageenan reacts with milk proteins to form a shear reversible gel. Gums such as cellulose, guar gum and xanthan gum are also used, especially in milk shake products where both body and foaming are desired. When the body of full fat milk has to be mimicked in the low fat/nonfat counterparts stabilizers play an important role. Reducing sugars and carbohydrates results in lowering total solids, hence a slightly higher level of stabilizers helps to improve mouthfeel of these products (Henderson 1971).

66.2.2 Different Flavored Milks

66.2.2.1 Chocolate Milk. The chocolate flavor of chocolate milk comes from the various sources of flavorings supplied by manufacturers in the form of cocoa, natural or Dutch chocolate, chocolate liquor, or chocolate syrup.

Chocolate milk is manufactured by addition of the various dry ingredients namely stabilizer, sweetener, to the cold milk and agitation continued for about 30 min until all the powder is dissolved. This is followed by batch or high-temperature short-time (HTST) pasteurization. Temperature is higher (generally 81.1°C or 178°F) than that used for fluid milk (generally 71.7°C or 161°F) because of added sugars. After pasteurization product must be cooled to 4.4°C (40°F) or lower as soon as possible. If sodium alginate is used as a stabilizer then it should be added to warm milk. If carrageenan is used as

the stabilizer then homogenization after addition of the powder is not recommended because of the possibility of settling. Carrageenan complexes with milk proteins keeping the cocoa particles in suspension. Typically kappa carrageenan is used in chocolate milks. For a thicker or a more shake like mouth feel gums such as guar or locust bean are also added to increase viscosity. Once a processing procedure is established it should be consistently followed to create an uniform product. Cocoa source and amount should be the same for uniformity in product color and flavor (Henderson 1971).

There are certain concerns of adding chocolate to milk. The calcium in the milk is bound to the oxalic acid in the chocolate resulting in an insoluble calcium oxalate complex, making calcium unavailable for absorption. Some children are allergic to chocolate or have skin problems aggravated by it (NDC 1995).

66.2.2.2 Natural Orange Juice Flavored Milk. A typical formulation is milk 56.8%, orange juice 38%, sugar 5%, and stabilizer 0.2% w/w. Milk (4°C) is stirred while the stabilizer and sugar dry mixture are added rapidly to reduce foaming. Stabilizers such as sodium carboxymethyl cellulose or propylene glycol alginate can be used. This milk stabilized mixture is then aged at 4°C for 15 min. Cold (4°C) orange juice is added just below the surface of the milk to minimize foaming. The natural orange juice flavored milk is then preheated, homogenized, pasteurized, cooled to 4°C, and packaged.

66.2.2.3 Banana Milk. Ripe bananas are peeled and pulp is pureed in a blender. The puree is quickly added to raw milk in a ratio of 1 : 5. The mixture is immediately warmed to 38°C, homogenized at 2000 psi, heated to 80°C for 15 s, and collected in sterile containers.

Banana puree develops a slightly brown discoloration while being prepared, but pasteurization of banana milk prevents further darkening. The brown discoloration in the banana puree has been reported to be inhibited by blanching banana puree, addition of antioxidants such as citric acid, ascorbic acid and potassium metabisulfite. In the final product food grade colors have also been reported to have been used (Rehman and others 1992, Ayub and Zeb 2002).

66.2.2.4 Mango Flavored Milk. Preparation of mango pulp preserve: Ripe mangoes are washed and peeled. Pulp is extracted using a pulper having a sieve size of about 40 mesh. Obtained pulp is heated to 85°C for 3–4 min.

Three parts of milk is mixed with one part of pulp (w/w). Sweetener is also incorporated. Stabilizer such as carrageen is optional. The mixture is blended to form a homogeneous mass. The homogenous mix is thermally processed at 90°C for 30 min (Ibrahim and others 1996).

66.2.2.5 Date Milk. Preparation of date syrup: Dates with half their weights in water are cooked in a pressure cooker for half an hour. Syrup strained and squeezed through cheese cloth. This is followed by concentration of date syrup by vacuum evaporation.

Milk is sweetened with sweetener (corn syrup or non caloric sweetener) and 3–5% date syrup is incorporated. Mixture is bottled or canned and retorted/sterilized at 120°C for 30 min (Yousif and others 1996).

66.3 FLAVORED MILK WITH ADDITIVES

Flavored milks are fortified with 10 essential vitamins and minerals as in case of MoonPie®. Caffeine as much as in cola is added to flavored milks and these flavored milks are

positioned as energy drinks by Hyper Cow™ for consumers of ages 15–25, typically upper-high school to graduate school students (Anon 2004).

66.4 FLAVORED MILK BASED BEVERAGES

Swerve, a milk beverage introduced by Coca-Cola is made with nonfat milk and provides 30% of the recommended dietary intake of vitamin A, vitamin C, vitamin D, and calcium. It is available in three flavors namely Vanana (combination of vanilla and banana), blooo (mix of blueberry and strawberry), and chocolate.

Swerve contains more than 50% milk and therefore carries the real seal, use of which is administered by the American Dairy Association. This product is available in 12 oz. cans and product is retort processed. Swerve is sweetened with sucralose and high fructose corn syrup which keeps calories low, to about 150 per can.

Raging cow, a milk beverage introduced by Dr Pepper/Seven Up Inc. (DPSU). It is available in five flavors namely, Chocolate Intensity, Pina Colada Chaos, Caramel Craze, Berry Mixed Up, and Jamocha Frenzy. Each of these flavored milk beverages contain four vitamins and minerals (Dahm 2004).

66.5 FLAVORED CARBONATED MILK BEVERAGES

The purpose of carbonation is to impart a unique sparkle or effervescence, enhance the flavor, mouthfeel and provide a refreshing aftertaste (Vinderola et al., 2000). Carbonation of milk also extends its shelf-life up to 8 weeks. Blueberry, strawberry, raspberry, peach, rootbeer, chocolate, and bubblegum flavored milks have been reported to be carbonated.

Typically the sweetener and stabilizer are added to the raw milk and pasteurized. The mix is then cooled to about 22°C and flavored. This complete mix is further cooled to 2°C for carbonation. Carbonation is conducted using a carbonator. Product is carbonated in the CO_2 pressure range of 0.35–1.5 kg/cm^2 (5–22 psi). Carbonated flavored milks are packaged in the CO_2 pressure range of 0.35–1.05 kg/cm^2 (5–15 psi) and refrigerated.

Carbonated blends of fruit juice and milk are produced in two flavors namely strange strawberry and odd orange, contain no added sugars and are fortified with calcium and marketed under the name of Freekee Soda by Britvic Soft Drinks. Other examples of carbonated beverages are e-Moo and Refreshing Power Milk (RPM). RPM comes in flavors such as vanilla cappuccino, Brazilian chocolate, and chocolate raspberry (Anon 2003a,b,c: 2004).

66.6 FLAVORED MILK POWDERS

The advantage of having flavored milk in powdered form is increased shelf and easy in bulk transportation of the product. Flavored milk powders are prepared using either spray drying or freeze drying.

66.6.1 Manufacture of Powdered Flavored Milks

66.6.1.1 Mango Milk Powder. A way to manufacture mango milk powder is as follows. Ingredients are taken in the following proportions. Fifty-two percent concentrated milk (30% solids), 14% cream (40% fat), 36% fruit pulp, 10% sugar, 0.4% stabilizer. The concentrated milk, cream and sugar are well mixed and preheated to 50°C followed by the addition of

sodium alginate and glycerol monostearate in a 1 : 1 ratio at rate of 1% total solids in mix. The heated mix is filtered, homogenized, pasteurized, and cooled. To this mix mango pulp is added and mixed. This mango milk mix is filtered and dried to obtain mango milk powder (Sharma and others 1974; Berry 2003; Dwyer 2003).

66.6.1.2 Banana Milk Shake Powder. One manner in which banana milk shake powder is manufactured is as follows. Ripe bananas are peeled, cut, blended with 1–1.5% distilled water. The puree is heated to 90°C for 10 min and cooled to 40°C. Pasteurized milk is condensed using a vacuum evaporator to 36% total solids and cooled to 40°C. A stabilizer (carboxy methyl cellulose) is dissolved in hot water at 60°C and added to this condensed cooled milk at the rate of 0.015% (w/w total solids). The stabilized condensed milk is then homogenized and mixed with the banana puree, homogenized again and spray dried. Ground sugar may be added to the spray dried banana milk shake powder (Laxminarayanana and others 1997; Paoletti and others 1992).

66.7 LOW CARBOHYDRATE FLAVORED MILKS

The recent advent of the Atkins low carbohydrate diet has sparked an interest among beverage manufacturers to lower their drinks in the carbohydrate contents. Reduced fat chocolate milk with 90% fewer carbohydrates than regular chocolate milk has been introduced by HP Hood under the "Carb Countdown" brand name. The Hood Carb Countdown dairy beverages are approved as a part of the Atkins Nutritional Approach™ eating plan, which is a low carbohydrate diet. An example of a lactose free milk is Refreshing Power Milk of Mac Farms.

Ultrafiltration is a membrane filtration technique which is characterized as having a molecular weight cut-off from 3000 to 100,000, the most common being 10,000 MW. Ultrafiltration rejects all the large milk components namely proteins, fats allowing the lactose, minerals, and water to pass through the membrane. It is here that with the addition of water or diafiltration even more lactose from the milk can be washed off. Use of ultrafiltration increases the percentage of protein in fluid milk as a method of fortifying proteins in fluid milk (Twiford 2004). This allows the flavor and mouth feel enhancing properties of milk proteins to be achieved naturally as opposed to adding nonfat dry milk which often leaves a cooked flavor in the fluid milk as well as increased sweetness from the excess lactose in NFDM. For the preparation of "low carb" or "carb free" dairy beverages ultrafiltration or a combination of ultrafiltration and diafiltration is used (Yau and others 1989; Lederer and others 1991).

66.8 FLAVORED MILKS AND NUTRITION

Each brand of flavored milk has its own formulation. The nutrient contribution of flavored milks are almost similar to their unflavored milk counterparts. Flavored milks are typically higher in carbohydrate content and hence provide more energy.

66.9 MYTHS ABOUT FLAVORED MILKS

There are several myths about flavored milks namely; nutritionally flavored milks are not similar to unflavored milks, flavored milks have a high sugar content, too much caffeine is

present in chocolate milk, flavored milks are not good for teeth. Putting the myths of flavored milks into perspective, both flavored and unflavored milks have about the same type and amount of nutrients such as protein, calcium, magnesium, phosphorous, riboflavin, vitamin A, vitamin B_{12}, as well as several other essential nutrients. Chocolate is the most popular flavored milk, has lesser sugar per 8 oz. serving than cola sodas. The amount of caffeine in chocolate milks is about the same as in decaffeinated drinks. High sugar foods not good for the teeth are particularly those that stick to the teeth. Being fluid, flavored milk are in contact with teeth for a short time, moreover calcium and phosphorous in flavored milks protect the teeth from decay (NDC 1995).

66.10 PACKAGING FLAVORED MILKS

Packaging involves materials, style, shape, special features, and graphics. Packaging influences purchase decisions, especially first-time trials.

What sets Raging Cow apart from other flavored milks and dairy drinks is the fact that it is the first milk beverage to be bottled on the recently FDA accepted commercial low acid aseptic plastic bottles. Raging Cow is unique with its closure, too. The flip top can be opened and closed with one hand, does not require the removal of the closure to remove the introduction seal and opens a full 220° to prevent the closure from touching the face of the consumer (Anon 2003a,b,c; 2004).

Milk Chugs™ are plastic, pints, quarts, 8 oz. multipack bottles. The highlight of this package is that it is resealable, has a flared cap that is easy to grip, and is a round, tapered, easy to hold bottle, which resembles the shape and size of old fashioned milk bottles. It is designed for portability, to suit the on-the-go consumer. It fits in the car cup holders, bags, coolers, and lunch sacks. Oberweis Dairy, North Aurora, IL, launched milk and coffee blends in single serve 12 oz. polyethylene terephthalate (PET) bottles (Anon 2003a,b,c; 2004).

Tetra Pak's LFA-20 filler for high density polyethylene (HDPE) bottles uses hydrogen peroxide gas to achieve package sterilization, unlike fillers for paperboard composite packages. LFA-20 filler is used to package Raging Cow milk drink and Slammers, line of flavored milk drinks (Anon 2003a,b,c; 2004; Higgins 2004).

Chocolate milk in aluminum cans competes in soft drink vending machines. The National Milk brand of chocolate milk are packaged in aluminum cans. The body of the can is first filled with chocolate-flavored milk. Twelve ounce cans are filled with only 11 oz. of flavored milk because retorting processing requires extra headspace. Containers are pressurized with liquid nitrogen gas and an aluminum end is seamed onto the open end of the can, sealing the can. This milk is then sterilized by the retorting process, which is essentially heating the contents of the can to a specified temperature for a predetermined time. The retorting temperature is typically 126.7°C (260°F). The goal of retort processing is to obtain commercial sterilization through the application of heat. This renders the product shelf-stable for an extended shelf-life. In this case, the chocolate milk has an ambient shelf-life of 12 months, which is about 11 months longer than traditionally pasteurized chocolate milk. Unlike traditional soft drink cans, which use color printing directly on the cans, the National Milk cans are unprinted. After retorting, roll-feed labels are cut and wrapped around the can. Cans convey through a heat tunnel, where labels shrink to fit the cans' counters. The image of a milkman holding an old fashioned bottle of fresh milk, is intended to communicate the product's nutritional value.

Byrne Dairy wanted to target the younger market. On their paperboard container they introduced a company mascot – a likeable, friendly-faced cow named "Byrnsie™" which was an instant hit with kids. NutraBrand Innovations Devon, PA introduced MegaMilk™ which is fat free milk that comes in three flavors namely, chocolate, strawberry, and vanilla – and is sold in pint-sized cartons with superheroes such as Batman and Superman on the package. Slammers flavored milks has various cartoon characters such as Tweety, Bugs Bunny, and superheroes such as Spiderman and Wolverine in action on their label. The label Swerve brand of flavored milks have a figure of a grinning cow wearing glasses, which also appeals to kids (Anon 2003a,b,c; 2004).

Extended shelf-life packaging is achieved primarily through filling in sanitary environments with packaging that has been sterilized. Hydrogen peroxide is the most commonly used sterilizing agent and packaging material is generally rinsed or blown dry with hepafiltrated air after sterilization. In some cases, plastic packaging can be blow molded with a sealed top using hepafiltrated air. The top is then trimmed in the filler just before filling. Both paperboard and plastic packaging materials can be manufactured with barrier layers that block light penetration and oxygen migration. This can be particularly beneficial for milk as milk tends to take on off-flavors when it suffers prolonged exposure to light or oxygen (Higgins 2004).

Multipack packaging is particularly suited to warehouse and club stores which are characterized by less frequent visits and larger consumer purchases. For fluid flavored milks this means everything from cases that hold two or more gallons, to cartons containing six, 12, or 24 single serve bottles (Anon 2004).

66.11 FUTURE OF FLAVORED MILKS

It would not be surprising in the future to see dairy-based beverages containing phytosterols and more of such health beneficial components incorporated.

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67

Fermented Milks Popular in Europe and North America

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67.1 Introduction/Background 411
67.2 Classification and Diversity of Products 412
67.3 Patterns of Production 413
67.4 Manufacture of Fermented Milks 413
67.4.1 Fermented Milks with Lactic Starter Cultures 415
67.4.1.1 The Use of Mesophilic Strains 415
67.4.1.2 The Use of Thermophilic Strains 417
67.4.1.3 The Use of Probiotic Strains 422
67.4.2 Fermented Milks with Yeasts and Lactic Starter Cultures 423
67.4.2.1 Kefir and Koumiss 423
67.4.2.2 Skyr 424
67.4.3 Fermented Milks with Mould and Lactic Starter Cultures 424
67.5 Conclusion and Future Trends 425
References 426

67.1 INTRODUCTION/BACKGROUND

While communities in the Middle East and Asia adopted fermented milks into their diets almost as soon as man began to domesticate animals, the inhabitants of Europe

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

developed a tradition of cheesemaking. Indeed, around 1000 AD, a number of varieties of cheese had been cited by early writers, and this domination of cheese over other dairy fermentations largely persisted until the early part of the 20th century (Robinson and Wilbey 1998). Some fermented milks did, of course, find favor with local populations, and buttermilk was one product that became popular in Scandinavia and, through immigration, in North America as well. However, it was not until 1910 when Metchnikoff published his work about the health promoting properties of yogurt that small sections of the general public began to take a serious interest in fermented milks (Räsić and Kurmann 1983; Tamime 2002b). In North America, acidophilus milk was marketed as beneficial health food; while in Russia, the traditional drinks of kefir and koumiss received more serious attention from the medical authorities (Alm 1991; Koroleva 1991).

However, it was not until the mid-1950s that various forms of sweetened and fruit-flavored yogurt went first on sale in urban areas in Switzerland. As figures of production rose (see Section 67.3), so manufacturers retained interest in yogurt by introducing an ever-wider range of fruit flavors. Around the 1990s, signs of the market saturation were beginning to appear, even though the per capita consumption in many Western countries remained comparatively low. The solution was provided by a resurgence of interest in "health-promoting" properties of cultures like *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus johnsonii*, and *Lactobacillus gasseri* along with various species of *Bifidobacterium*. In addition, it was noted that these probiotic cultures are much less acid tolerant, and even in the presence of *Streptococcus thermophilus*, the acidity of "probiotic or bio-yogurts" rarely exceeds 1 g/100 mL. The resultant products are, therefore, more mild in flavor and, as a consequence, a whole new group of consumers emerged who, irrespective of their views about the value of probiotic cultures, enjoyed the sensory properties of this new range of fermented milks.

Nevertheless, the retail value of the yogurt market in the United Kingdom was around £814 million (Anon 2003a), in the United States was US $2396 million (Jelen and others 2003), and such figures are common place around the world.

67.2 CLASSIFICATION AND DIVERSITY OF PRODUCTS

The "traditional" lactic acid bacteria used in fermented milks belong to the genera of *Lactococcus*, *Leuconostoc*, *Streptococcus*, and *Lactobacillus*; the former two genera are mesophilic, whilst the latter types are thermophilic (Campbell-Platt 1987; Kurmann and others 1992). Examples of probiotic microorganisms are *Lactobacillus* spp., *Pediococcus acidilactici*, *Bifidobacterium* spp., *Enterococcus* spp., and *Saccharomyces boulardii*. Many yeasts species have been identified in kefir grains from different countries, and for more details refer to the review by Tamime and Marshall (1997); however, only one mold (i.e., *Geotrichum candidum*) is used in viili production.

Nevertheless, taking into account the flora that dominate in these products, Robinson and Tamime (1990) classified fermented milks based on the main metabolites of the starter cultures (e.g., type of organic acid and carbonyl compounds produced, presence of carbon dioxide in the gel and/or production of ethanol), and Table 67.1 illustrates how some products fall within such a scheme of classification.

TABLE 67.1 A Scheme of Classification of Fermented Milk Products.

Type of Fermentation	Example of Products	Microorganisms
Lactic		
Mesophilic-type	Buttermilk	*Lactococcus* spp. and *Leuconostoc* spp.
	Tätmjölk	*Lactococcus* spp. including ropy strains
	Dickmilch	Similar to buttermilk + yeast
Thermophilic-type	Yogurt, zabadi	*Lactobacillus delbreuckii* subsp. *bulgaricus* + *Streptococcus thermophilus*
	Bulgarian buttermilk	*Lb. delbreuckii* subsp. *bulgaricus* (on some occasions *S. thermophilus* or cream culture may be present)
Probiotic-type	ABT, Philus	*Lactobacillus acidophilus* + *Bifidobacterium lactis, animalis* or *bifidum* + *S. thermophilus*
	ACT4	Similar to ABT + *Lactobacillus casei*
	Actimel	Yogurt + *Lb. casei* (strain Imunitass)
	Gaio	*S. thermophilus* + *Enterococcus faecium*
	BRA	*Bifidobacterium infantis* + *Lactobacillus reuteri* + *Lb. acidophilus*
	Yakult	*Lactobacillus casei* (strain Shirota)
	Aktifit	Similar to ABT + *Lactobacillus rhamnosus* GG
Yeast-lactic	Kefir	Many LAB[a] + lactose-fermenting yeasts + *Acetobacter aceti*
	Koumiss	*Lb. delbreuckii* subsp. *bulgaricus* + *Lactococcus* spp. + yeasts + *A. aceti*
Mold-lactic	Viili	Similar to buttermilk + *Geotrichum candidum*

[a]LAB = lactic acid bacteria.
Source: Data compiled from Kurmann and others (1992), Tamime and Marshall (1997), and Tamime (2002a, 2002b).

67.3 PATTERNS OF PRODUCTION

There are no data available worldwide in the dairy industry to indicate the true global production of every type of fermented milk produced each year in any one country. Tables 67.2 and 67.3 show annual production figures of these products in some selected countries, and the overall pattern of production over the past few decades is steadily increasing. As mentioned earlier, the popularity of fermented milks in many countries may be due, in part, to tradition; especially in countries of the Balkans, East European countries, and the Middle East, the incorporation of fruit in yogurt and/or the health promoting properties associated with "nontraditional" microorganisms. Nevertheless, the principles underlying the various aspects of manufacture have altered little with time, and an understanding of these basic tenets is essential for efficient control at plant level.

67.4 MANUFACTURE OF FERMENTED MILKS

As mentioned elsewhere, there are now numerous types of fermented milk manufactured in different parts of the world that could be classified as gel/liquid, concentrated/strained, frozen, or dried. Recent publications dealing with the major types of yogurt and related products include those by Tamime and Marshall (1997), Tamime and Robinson (1999), Tamime and others (2001), Robinson and others (2002), Jaros and Rohm (2003) and Anon. (2003b).

TABLE 67.2 Total Production (×1000 tonnes) of Milk Drinks and Fermented Products Including Yogurt.

	Year				
Country	1975	1985	1996	2000	2001
Australia	13.9	44.0	101.0	108.0	NR[a]
Austria	54.2	66.7	113.0	173.0	170.0
Belgium	37.0	59.4	254.0	216.0	NR
Canada	16.2	63.1	100.0	150.0	176.0
Czechoslovakia	45.2	86.7	113.0/24.0[b]	142.0/63.0[b]	NR/NR[b]
Denmark	65.7	79.4	136.0	145.0	219.0
Federal Germany	544.0	548.0	1882.0[c]	2290.0[c]	2180.0
Finland	166.3	183.0	198.0	210.0	211.0
France	414.2	698.6	1484.0	1682.0	1253.0
Iceland	NR	4.9	NR	10.0	10.0
Israel	48.4	67.8	135.0	179.0	NR
Netherlands	193.4	262.0	721.0	713.0	691.0
Norway	36.1	58.6	73.0	75.0	91.0
Poland	110.0	34.5	208.0	575.0	223.0
Spain	122.4	203.0	500.0	620.0	NR
Sweden	163.2	228.3	251.0	400.0	303.0
Switzerland	71.0	107.3	NR	167.0	NR
United Kingdom	67.3	173.0	NR	NR	NR

[a]Not reported.
[b]Data for Czech Republic and Slovakia, respectively.
[c]Data includes German Democratic Republic.
Production data for fermented milks in countries in the Middle East, North Africa, the Balkans, and the rest of the world (i.e., countries not listed in Tables 67.2 and 67.3) are not available.
Source: Data compiled from IDF (1977, 1987, 1999, 2002, 2003).

TABLE 67.3 The Pattern of Total Production (×1000 tonnes) of Milk Drinks and Fermented Products Including Yogurt in Some Selected Countries.

	Year				
Country	1998	1999	2000	2001	2002
Argentina	224	250	243	240	228
Belarus	342	375	309	NR[a]	NR
China	120	130	270	420	530
Croatia	37	71	NR	NR	NR
Cyprus	Nr	Nr	Nr	8	8
Estonia	NR	9	8	9	9
Hungary	95	110	125	117	121
Jordan	47	56	NR	NR	NR
Mexico	NR	Nr	370	381	387
Thailand	300	620	650	NR	NR
South Africa	138	140	142	146	148
Ukraine	170	172	174	NR	NR

[a]Not reported.
Production data for fermented milks in countries in the Middle East, North Africa, the Balkans, and the rest of the world (i.e., countries not listed in Tables 67.2 and 67.3) are not available.
Source: Data compiled from IDF (2001, 2002, 2003).

The quality of all fermented products is governed by the compositional and microbiological qualities of the raw milk, added ingredients, processing of the milk base and handling the coagulum after the fermentation stage. The main difference of set- and stirred-type products is the nature of the gel structure; however, in the former type, the processed milk is fermented in the retail container, whilst the stirred-type is fermented in bulk in large tanks. Nevertheless, the manufacturing stages of all fermented milk products are broadly similar, and the process parameters of these operations have been detailed by Tamime and others (2001) and Anon. (2003b), and in the present context, details of the manufacturing stages of some industrialized fermented milk products are discussed below.

67.4.1 Fermented Milks with Lactic Starter Cultures

These fermented milks are the most popular types in the dairy industry worldwide (see Table 67.1), and some examples are as follows.

67.4.1.1 The Use of Mesophilic Strains. Blends of the microflora of the genera *Lactococcus*, *Leuconostoc*, and *Pediococcus* are commonly used during the manufacture of products denoted as "mesophilic" lactic acid fermentations, and this term is used for strains whose growth optima is between 20°C and 30°C.

Buttermilk. At present, traditional or natural buttermilk is not commonly manufactured in large factories, as it is the by-product of butter making after churning ripened or cultured cream. However, the industrialized product is currently known as "cultured" buttermilk. This fermented milk is made from skimmed milk that has not been fortified and heated to ~90°C for up to 20 s, cooled to 60°C, and homogenized at 18–20 MPa, cooled and fermented at 20°C with a mixture of mesophilic lactic acid bacteria known as DL culture (see Table 67.1). After reaching the desired acidity, the gel is thoroughly stirred, homogenized at 5–10 MPa pressure and 20°C, cooled to 4°C, and packaged. The primary objective of postfermentation homogenization of the gel is to smooth the product without any lumps (Tamime and others 2001). Furthermore, deaeration of the milk before the heat treatment stage is highly recommended during the manufacture of cultured buttermilk and closely related products because defects such as lumpiness and granules in the gel will be minimized.

In some countries, for example the United States, the total solids (TS) content of buttermilk may be fortified up to 11.4 g/100 g and the fat content may range between 0.25 and 1.9 g/100 g. In Poland, fortification of the milk base of buttermilk is practiced, while in Scotland buttermilk may have 13.33 g/100 g TS and 0.2 g/100 g fat (Muir and others 1999; Tamime and others 2001).

Some advances in the technology of cultured buttermilk may include progress in different scientific fields, and the following papers are recommended for further reading (Klaver and others 1992; de Vos 1993; Butler and McNulty 1995; Heiler and Schieberle 1996, 1997a,b; Roushdy and others 1997; Tamime and Marshall 1997; Butler and O'Donnell 1999; Ervol'der and others 1999; Tuitemwong and others 2001; Rodas and others 2002).

Scandinavian Sour Milk Products. In Norway, Sweden and neighboring countries, traditional buttermilks that are slimy or ropy are widely produced and herbs or grasses are added to the milk (i.e., by rubbing the interior of the milk pails) to produce thick fermented

milks. The products are known as tätmjölk, täetmjölk, filmjölk, tåttfil, långjölk, and filbunk, and the fermentation relies on the indigenous undefined mesophilic microflora present in the milk. At present, mixed cultures of *Lactococcus* spp., including the ropy variants, is used to manufacture these products in large factories in a manner similar to cultured buttermilk. However, filbunk is made from whole milk and consumed immediately. In some old scientific publications, the starter culture used was known as *Bacterium laticus longi* and, at present, some scientists have suggested the name *Lactococcus lactis* biovar *longi* as a variant strain of *Lactococcus* spp. capable of producing exopolysaccharides (EPS) (Tamime and Marshall 1997).

In the commercial production of filmjölk, whole milk is standardized (i.e., fat content ranging from 0.5 to 3 g/100 g), preheated to 70°C, homogenized at 17.5–20 MPa, deaerated, heated to 90–95°C for 3–6 min in an external holding tube section of the plate heat exchanger, and cooled to 20°C. The starter culture is composed of a mixture of *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris* (i.e., the acid producer), *Lc. lactis* biovar *diacetylactis*, and *Leu. mesenteroides* subsp. *cremoris* (i.e., flavor and aroma producer). The milk is fermented for ~20 h at 20°C (titratable acidity ~0.9 g/100 mL), agitated, cooled and finally packaged (Anon. 1987).

Ymer. This is a Danish concentrated fermented milk and may contain (g/100 g) solids-not-fat (SNF) 11 including the protein 5–6 and fat 3.5. The starter culture used is similar to buttermilk, and a similar product, lactofil, is made in Sweden.

Currently in Denmark, the ultrafiltration (UF) process is used to produce all the ymer commercially. Skimmed milk or possibly standardized milk is heated, UF at 50°C (i.e., SNF 11 g/ 100 g), homogenized at 23 MPa and 65°C, heated to 90°C for 5 min and fermented with a buttermilk starter culture until pH 4.5. After heavy stirring, blending with cream (36 g fat/100 g), cooling in a plate heat exchanger to 12°C, the product is packaged and transferred to a cold store (Bøejgaard, personal communication). Alternatively, ymer could be made using nozzle separators similar to quarg, concentrated yogurt or skyr (Tamime and Marshall 1997; Tamime and Robinson 1999; Tamime and others 2001; Figs 67.1 and 67.3).

Cultured Cream. This fermented milk product has a pleasant acidic taste, "buttery" aroma and nutty meat-like flavor (Tamime and Marshall 1997). The manufacturing stages can involve standardization of cream to fat content (low-fat cream to 10–12 g/100 g or high-fat cream to 20–30/100 g), preheating to 70°C, homogenizing at 15–20 MPa (low-fat cream) or 10–12 MPa (high-fat cream), heating to 90°C for 5 min, cooling to 20°C, inoculation with mixed starter cultures similar to those used for filmjölk making, and fermentation for 18–20 h to an acidity of 0.8 g/100 mL (Tamime and Marshall 1997; Folkenberg and Skriver 2001; Anon. 2003b). After fermentation, the cultured cream is cooled quickly and packaged. However, a slight reduction in viscosity will occur due to mechanical handling of the product (i.e., cooling, pumping, and packaging), and for this reason some manufacturers produce a set-type product or employing the addition of stabilizers (optional) (Lee and White 1993).

Optional processes in cultured cream technology may include: (1) the addition of citric acid to the cream before processing to enhance the metabolic activity of the starter culture, (2) postfermentation heat treatment and followed by homogenization and hot packaging; a process similar to the manufacture of cream cheese, (3) the use of a probiotic microflora with the starter culture (Tamime and Marshall 1997), and (4) a reduction in syneresis of

cultured cream was achieved using EPS microfloras and *S. thermophilus* with the mesophilic starter culture (Adapa and Schmidt 1998).

67.4.1.2 The Use of Thermophilic Strains. The majority of fermented milks consumed in Europe and North America are manufactured with species of bacteria with growth optima of 35–45°C, and this characteristic derives from the fact that the species in question are either of intestinal origin, e.g., *Lb. acidophilus* or *Bif. bifidum*, or evolved in the Middle East where the ambient temperature in the summer is often well in excess of 35°C, for example, *Lactobacillus delbreuckii* subsp. *bulgaricus* and *S. thermophilus*. However, beyond sharing certain morphological similarities and a tolerance of temperatures much higher than those associated with mesophilic strains, the two groups differ quite markedly, especially with respect their behavior in milk. Consequently, the role of the two groups in the manufacture of retail products is best considered separately.

Yogurt. The continued use of *Lb. delbreuckii* subsp. *bulgaricus* and *S. thermophilus* for the manufacture of yogurt was, at least initially, historical in origin, but there are good reasons for continuing with the tradition, for when growing in milk, the two organisms interact synergistically. This interaction depends upon two main facts. First, *S. thermophilus* grows more rapidly than *Lb. delbreuckii* subsp. *bulgaricus* in milk, and ferments lactose homofermentatively to give L(+) lactic acid as the principal product. Carbon dioxide is also liberated by the breakdown of urea in the milk by urease and, usually, formic acid (up to 40 μg/mL). All three metabolites stimulate the growth of *Lb. delbreuckii* subsp. *bulgaricus*. Second, Although some free amino acids occur naturally in milk or are released during the heat treatment, the level of glutamic acid, histidine, cysteine, methionine, valine, or leucine, for example, are not present at levels sufficient to support extensive growth of *S. thermophilus*. However, *Lb. delbreuckii* subsp. *bulgaricus* can hydrolyze casein – especially β-casein – by means of a wall-bound proteinase to release polypeptides and, as *S. thermophilus* can readily hydrolyze these peptides, the free amino acids that are essential for further development of both species become available.

The practical result of this synergy is that both species grow rapidly and actively metabolize sufficient lactose to lactic acid to complete the fermentation of milk to yogurt within 3–4 h using an active bulk starter culture – one species alone might take 12–16 h to produce the same level of acidity. In addition, metabolites liberated by the two species give yogurt a flavor that is distinctly different from any other fermented milk. Acetaldehyde levels up to 40 mg/kg are the major component of the flavor profile (Marshall and Tamime 1997). Some strains of the two species can also produce EPS material, such as glucans, or polymers involving glucose, galactose, and rhamnose as the constituent sugars (Sikkema and Oba 1998; Laws and Marshall 2001; Laws and others 2001). The presence of these metabolites enhances considerably the viscosity/consumer appeal of the retail yogurt.

Bovine milk is usually the base material for making yogurt in the Western world, although ovine, caprine, or buffalo milks can also be employed. The fat content of bovine milk tends, depending on the breed of cow, diet, and stage of lactation, to be in the range of 3.0–4.5 g/100 g, and this value has to be adjusted according to consumer taste and/or market demand; the fat content of retail yogurt can range from 0.1 to 4.5 g/100 g. However, the critical feature of any milk is the level of SNF which, in bovine milk varies from 8.5 to 9.0 g/100 g according to the season of the year, with

around 4.5 g/100 g being lactose and 3.3 g/100 g being protein (2.6 g/100 g casein and 0.7 g/100 g whey proteins); these proteins, together with minerals like calcium and phosphorous, give rise to the basic gel structure of the product.

Today, various levels of SNF (g/100 g) are sought by manufacturers but, in general, the recognised categories are: drinking yogurt 11, cheap/cooking yogurt 12, standard base for stirred yogurt 13–14 or top-of-the range, natural set yogurt 17–18.

On an industrial scale, this elevation of the SNF can be achieved by using single-stage evaporator (EV) or UF at 70°C and 50°C, respectively. Both processes remove water and, hence, raise the levels of both residual fat – assuming that prior separation took place – and SNF in the milk base, but UF does allow some losses of lactose and minerals (Grandison and Glover 1994). In terms of product quality, either process is acceptable and, while the final choice may depend on the cost and/or availability of process plant, some data suggest that EV gives rise to a smoother coagulum, whilst the UF produces a firmer yogurt (Lankes and others 1998). The alternative route is to add skimmed milk powder to the liquid milk at ~40°C, and systems of hoppers and high speed blenders (e.g., some units operate completely under vacuum and deaeration of the reconstituted milk is not required) can be employed to ensure full and rapid incorporation of the milk powder.

Once the desired level of SNF has been achieved the milk will be usually be homogenized at 70°C. Heat treatment follows, and at temperatures well above normal pasteurization of market milk. One of two alternative systems is usually employed, and while one involves passing the milk base through a plate heat exchanger with a holding-tube of sufficient capacity to raise the temperature to 90–110°C for up to 5 min, the other system necessitates heating the milk in a process vessel to 80–85°C with a holding time of 30 min. The former system is the treatment of choice for large-scale operations. This heating over an extended period of time alters the physicochemical properties of the caseins and denatures the whey proteins and, as a result, β-lactoglobulin becomes attached to the κ-casein, so improving the texture (set yogurt) or viscosity (stirred yogurt) of the final product. Other essential actions of the heating stage are: (1) a partial breakdown of the whey proteins to amino acids that stimulate the activity of the starter culture; (2) an expulsion of oxygen from the milk that is beneficial for the growth of the micro-aerophilic starter bacteria; and (3) a reduction in the indigenous microflora in the milk that might otherwise compete against the added lactic acid bacteria.

After the heat-treatment stage, the milk will be cooled to 42–43°C ready for the addition of the starter culture consisting of a 50 : 50 mixture of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. In general, the milk will be cooled in a plate heat exchanger and transferred at the desired temperature to an insulated fermentation vessel of 5–10,000 L capacity, but smaller dairies may carryout the fermentation in same vessel previously employed to heat-treat the milk (i.e., batch process). How the culture is added to the milk will depend upon its physical form, that is, a liquid culture prepared in the diary (anticipated addition rate of 20 mL/L of process milk), or a concentrated freeze-dried or frozen purchased for direct inoculation into the processed milk (Tamime 2002a; Anon. 2003b).

Once the milk has been inoculated, it will follow one of the two routes illustrated in Figure 67.1 involving incubation chambers or fermentation tanks. In the case of set yogurt, the retail cartons are "palletized" and transferred to a special tunnel. The tunnel is divided into two sections and, while warm air at 42°C is blown through the first part, the second section acts as a "cooler.") The pallets of yogurt cartons move through the

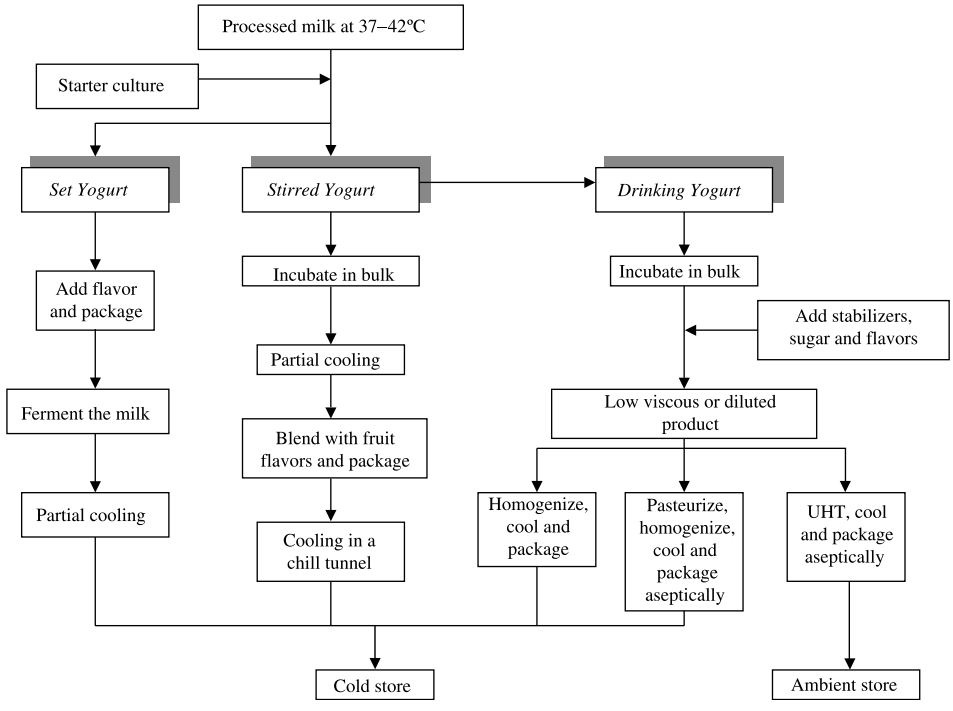

Figure 67.1 *Flow chart showing the processing stages of set, stirred and drinking yogurts. Adapted from Tamime and Robinson (1999) and Anon. (2003b).*

tunnel on a conveyor belt and, although the warm coagulum is in motion during the fermentation period, minimum structural damage is achieved by fitting smooth rollers.

During the manufacture of stirred yogurt, the milk is fermented in bulk in special incubation tanks (Tamime and Greig 1979; Tamime and Robinson 1999). There are two main types of tank in commercial use, namely insulated fermentation tanks which are used only as "incubators," and multipurpose tanks, that is, jacketed tanks that are used for all stages of yogurt production.

As the fermentation begins, the population of *S. thermophilus* develops rapidly (Robinson 2000). Over the next 2 h, however, the synergistic influence of the streptococci encourages more rapid growth and metabolism in *Lb. delbrueckii* subsp. *bulgaricus* and, after 4 h, the populations of each starter organism may well exceed 2.0×10^7 cfu/mL. The other result of this microbial activity is that the acidity of the milk will have risen to around 1.0–1.2 g/100 mL lactic acid (around pH 4.2–4.3).

At this acidity – which is probably the maximum level acceptable to consumers of stirred fruit yogurt in the West, the milk proteins will have coagulated to form a firm gel (Lucey and others 2000). Further acidification may cause the protein gel to shrink and expel whey to the extent that it can become a definite fault in set yogurts.

Final processing, for example, for set yogurt fermented in retail cartons, cooling is usually achieved by blowing cold air through the incubation room, but it is important that the rate of cooling can affect the structure of the coagulum. Excessive chilling rates can lead to whey separation due to contraction of the protein filaments, which, in

turn, affects their hydrophilic properties (Rasic and Kurmann 1978). However, a typical cooling rate of set yogurt using a continuous cooling tunnel is as follows: (1) temperature drop to 35°C should be reached within 30 min, (2) a further drop in temperature to 18–20°C in another 30–40 min, and (3) final cooling to <5°C takes place in the refrigerated cold store (Anon. 2003b).

Cooling of the base for stirred yogurt requires the circulation of chilled water (2°C) through the jacket (i.e., in-tank cooling system) of the vessel, or pumping the warm yogurt (42°C) through a plate or tubular cooler to cool the yogurt base to 20–25°C before mixing it with fruit/flavors and packaging. For details of the different packaging systems available, for example, cup fillers, form-fill-seal units and carton fillers refer to the publications by Robinson and Tamime (1993) and Tamime and Robinson (1999). Final cooling to 5°C takes place in a chill tunnel (i.e., quick cooling) and/or refrigerated cold store. However, when using a chill tunnel, the temperature drop of the packaged and palletised yogurt cups is from 20°C to <4°C in few minutes (Tamime and others 2001).

Bulgarian Buttermilk. This product is mainly produced in Bulgaria, and the manufacturing stages are similar to yogurt. Unlike the more popular cultured buttermilks fermented with mesophilic cultures, Bulgarian buttermilk is produced by *Lb. delbrueckii* subsp. *bulgaricus* acting alone (Tamime and Marshall 1997). Pasteurized whole milk provides the substrate for the fermentation, and incubation at 40–42°C for 12–16 h is employed to achieve the desired level of acidity. It is reported (Tamime and Marshall 1997) that the product has a flavor reminiscent of yogurt, that is, a flavor dominated by acetaldehyde. However, on some occasions *S. thermophilus* or a cream culture could be added to *Lb. delbrueckii* subsp. *bulgaricus* (Robinson and others 2002).

Drinking Yogurt. This product is really a stirred yogurt with low viscosity (i.e., ~11 g/100 g) and it is flavored with fruit juices, synthetic flavors, coloring compounds, and in some instances stabilizers are added to increase the viscosity of low solids fermentate. Traditionally, in some countries a whole- or low-fat fermented milk is diluted with either water, cheese whey, or whey from the manufacture of concentrated yogurt (see subsequent section), and the mixture is homogenized to improve the stability of the milk solids content in the aqueous phase followed by heat treatment to extend the shelf-life of the product.

Three different types of commercially produced drinking yogurt may be marketed, and these are illustrated in Figure 67.1 (Robinson and others 2002; Anon. 2003b). In brief, the fermented milk is processed as follows: (1) short shelf-life product, for example, 2–3 weeks at refrigerated temperature – the fermentate is homogenized, cooled, and packaged; (2) medium/extended shelf-life product, 1–2 months at refrigerated temperature – the fermentate is heated, homogenized, cooled, and aseptically packaged; and (3) long shelf-life product, for example, several months at room temperature – similar to the process described in (2), but the fermentate is ultraheat treated (UHT).

Concentrated Yogurt. This type of concentrated product (e.g., labneh, laban zeer, and mastou in the Arab countries, tzatziki in Greece, or Greek-style in the United Kingdom is manufactured from plain/natural, stirred-type yogurt, and the synonyms for closely related products that are produced in many countries have been reviewed recently by Tamime and Robinson (1999) and Robinson and others (2002). For example, ymer and chakka/shrikhand (in Denmark and India, respectively) are derived from milk fermented with a mesophilic starter culture, while the microflora of skyr (in Iceland) consists of a

yogurt starter culture, *Lactobacillus helveticus* and a lactose-fermenting yeast (for further details refer to section 67.4.2.2, and Puhan and others 1994).

Traditionally, most of these concentrated products were manufactured using the cloth bag method, which will not be reviewed, as it is not widely used in Europe and North America. At present, the traditional process has been mechanized using one of the following techniques, that is, nozzle separator, membrane filtration, or product formulation. The latter method will not be reviewed as the milk (i.e., 24–26 g/100 g total solids containing ~10 g fat/100 g) is handled and processed in a similar way to that used for the production of set-type yogurt.

Two systems, that is, mechanical separators (also known as quarg or nozzle separators) and UF, are available to manufacture concentrated or strained yogurt; these processes are similar to those used for the production of quarg. Figure 67.2 illustrates the manufacturing stages of concentrated yogurt where two options are available for production. In either process the fermented skimmed milk is stirred vigorously, thermized at 57°C for 1–2 min (Tamime and others 2001) or at 65°C for 30–300 s (Bøejgaard, personal communication), passed through a duplex filter to remove any large clots, cooled to 40–45°C, concentrated to 15–18 g solids/100 g, partially cooled, standardized with cream or blended with fruit (optional) and, finally packaged. However, in the second option (i.e., extended

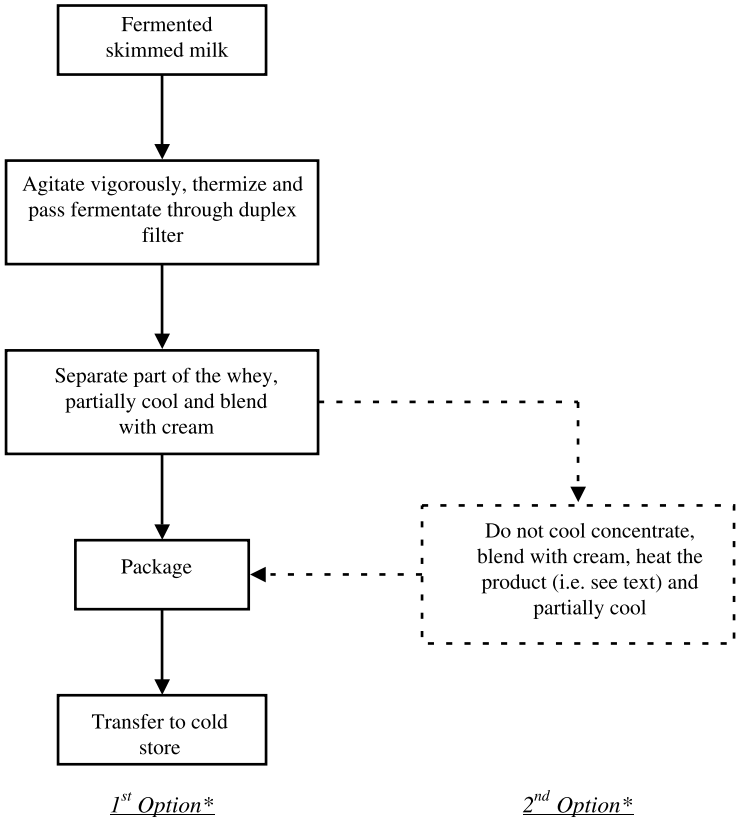

Figure 67.2 *Flow chart for the manufacture of strained/concentrated yogurt. Refer to text for more details.*

shelf-life concentrated yogurt), the fat standardized product is blended and vigorously mixed with a suitable stabilizer (optional) in the buffer tank, heated to 70°C, partially cooled, packaged, and finally transferred to the cold store.

The process control of the nozzle separator is governed by: (1) model type and size of the separator, (2) number and diameter of the nozzles, (3) temperature of separation, (4) concentration factor, and (5) the solids content in the whey; these aspects have been detailed by Tamime and others (2001). However, recent developments in the design of the nozzle separators have made it feasible to use fermented whole milk for the production of concentrated yogurt, but no published data are available (Tamime 2003).

The UF system has been also used for the production of concentrated yogurt, and the process line is similar to those shown in Figure 67.1, but UF modules replace the nozzle separator. Review of the processing conditions using the UF technology during the manufacture of concentrated yogurt have been reported by Tamime and Robinson (1999), Tamime and others (2001), and Tamime (2003).

67.4.1.3 The Use of Probiotic Strains. The word "probiotic" was defined by Fuller (1989) as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance." This definition stressed the requirement of viability of the probiotic cultures and introduced the aspect of a beneficial effect on the host and, today, this definition of probiotic is the most generally used (Charteris and others 1998; Fooks and others 1999); however, other definitions of probiotic have been reviewed by Gardiner and others (2002).

In practice, fermented milks are excellent vehicles for the transfer of selected strains to humans (Tamime 2002a,b), and a wide selection of lactic acid bacteria have been used in probiotic products around the world (see Table 67.4).

While milk drinks are popular some societies, "spoonable" yogurt-like products have acquired the major share of the probiotic dairy products market in the West (Robinson 2000). Consequently, manufacturers of therapeutic products have concentrated on the production of so-called "bio-yogurts" – Codes of Practice or Legislation curtail the use of the term "yogurt" as *Lb. delbrueckii* subsp. *bulgaricus* is not the dominant culture involved.

TABLE 67.4 The Main Species of Microorganisms that have been Used as Human Probiotics.

Lactobacillus spp.	*Bifidobacterium* spp.	*Enterococcus* spp.	*Saccharomyces* spp.
Lb. acidophilus	*Bif. adolescentis*	*Ent. faecalis*	*S. boulardii*
Lb. amylovorous	*Bif. animalis*[a]	*Ent. faecium*	
Lb. casei	*Bif. breve*		
Lb. crispatus	*Bif. bifidum*		
Lb. fermentum	*Bif. infantis*		
Lb. gallinarum	*Bif. lactis*[a]		
Lb. gasseri	*Bif. longum*		
Lb. johnsonii			
Lb. plantarum			
Lb. salivarius			
Lb. reuteri			
Lb. rhamnosus			

[a]Some *Bif. lactis* strains are synonymous for *Bif. animalis* and vice versa.
Source: Tamime (2002b) Itsaranuwat and others (2003).

As mentioned earlier, probiotic products must, to provide any benefit for the consumer, contain high counts of both *Lb. acidophilus* (or other recognized species – see Table 67.4) and *Bifidobacterium* spp., with "high" meaning above the "therapeutic minimum" of 1.0×105 cfu/mL for each organism (Robinson and Samona 1992). Thus, *Bifidobacterium* spp., as with the *Lactobacillus* spp., have a distinct role to play in the ecology of the human intestine, and members of the both genera have been positively identified as benefiting specific groups of patients (MacFarlane and Cummings 2002; Ouwehand and others 2002).

67.4.2 Fermented Milks with Yeasts and Lactic Starter Cultures

These are alcoholic milk beverages, and some typical examples, such as kefir, koumiss, acidophilus-yeast milk, and acidophiline, are mainly produced in northeast Caucasian mountains, in the Steppes, Mongolia, and former USSR. Only kefir has been commercially developed, and a limited volume is produced in Western Europe and the rest of the world. The following papers are recommended for further reading regarding aspects of microbiological quality, processing conditions, and nutritional and "functional" or neutraceutical properties of these products (Tamime and Marshall 1997; Kilic and others 1999; Michelli and others 1999; Muir and others 1999; Assadi and others 2000; Guzel-Seydim and others 2000a,b, 2003; Özer and Özer 2000; Jankowska and others 2001; Lourens-Hattingh and Viljoen 2001; Mainville and others 2001; Beshkova and others 2002; Frengova and others 2002; Robinson and others 2002; Simova and others 2002; Verachtert 2002; Anon. 2003b; Cheirslip and others 2003; Paraskevopoulou and others 2003; Farnworth and Mainville 2003).

67.4.2.1 Kefir and Koumiss. Kefir is made using milk of different species of mammals (e.g., cow, sheep, or goat), whilst koumiss is traditional made from mare's milk; however, the latter product will not be reviewed since the production system is not commercialized. Kefir has a lactic/sour taste, a foaming and effervescent characteristic as a result of CO_2 production and ethanol content as high as 2% depending on the yeast species present in the starter culture (i.e., in the form of grains). The microflora of the starter cultures for these products is not well defined, and may contain lactic acid bacteria (*Lactococcus*, *Lactobacillus*, *Leuconostoc* species, and *S. thermophilus*), *Acetobacter* spp., yeasts (*Saccharomyces*, *Kluyveromyces*, *Candida*, and *Torulaspora* species) and possibly *Geotrichum* spp. (Witthuhn and others 2004).

The commercial preparation of the kefir milk base is similar to yogurt making (i.e., preheating to 65–70°C, homogenization at 17–20 MPa and heating to 90–95°C for 5 min) and followed by cooling the milk to 22–25°C, which is the incubation temperature; however, the subsequent stages of manufacture are as follows.

First, the traditional process relies on seeding the processed milk base at ~22°C with kefir grains (2–10 g/100 mL) and fermenting for 12–24 h; on the following day, the grains are separated, washed in cold water and reused subsequently to produce more kefir (Schoevers and Britz 2003). The fermentate is cooled to ~10°C, blended with fruit (optional), packaged and stored at 5°C. Traditionally, glass bottles are used to package kefir as they can withstand the CO_2 pressure generated by the yeast; alternatively, semirigid containers may be used when lower volumes of CO_2 is produced in the kefir, but the container may lose its shape (i.e., bulge) due to the pressure that still exists inside the package.

Second, for commercial-scale operation, Koroleva (1991) developed a process in which kefir grains were used to ferment the milk and, after separating the grains, the fermentate (i.e., referred to as a bulk starter culture) was added to the processed milk base at 23°C at a rate of 3.5 mL/100 mL, incubated at 23°C for 20 h, cooled ~15°C, and "ripened" for 12 h allowing the yeast to grow and ensuring maximum stability of the coagulum. The subsequent stages of manufacture are similar to those described during the production of traditional kefir. Currently, starter cultures companies, such as Rhodia Foods and Danisco, have developed defined kefir starter cultures (e.g., freeze-dried) that can be used either for direct-to-vat inoculation (DVI) for the production of kefir or as a bulk starter culture. The latter type of starter culture is used to inoculate the processed milk at ~22°C; however, by using either DVI or kefir bulk starter, the "ripening" stage is not required and no kefir grains are produced in the final product. The properties of such types of kefir using different milks and starter cultures (i.e., grains, DVI, and bulk starter culture) were recently reported by Wszolek and others (2001).

67.4.2.2 Skyr. This is an Icelandic, concentrated, fermented skimmed milk product that is produced commercially using the cloth bag method (i.e., traditional process) or a nozzle separator similar to the production of quarg in Germany. The total solids content ranges between 17.5 and 20.8 g/100 g for the mechanized and the traditional skyr, respectively (Tamime and Marshall 1997).

This fermented milk product is only produced in Iceland and, in the late 1980s, one of us (AYT) was involved in the technology transfer of skyr to Scotland. A frozen skyr starter culture was brought from Iceland that consisted of *S. thermophilus*, *Lb. delbreuckii* subsp. *bulgaricus*, *Lb. helveticus*, and a lactose-fermenting yeast; however, some authors have reported that the microorganisms in the skyr starter culture consist of *Lactococcus* spp., *Lb. casei*, and yeast (Fondén and others 2003). The commercial method of production of natural skyr using the nozzle separator is shown in Figure 67.3 and can be summarized as follows. Skimmed milk is fortified with diluted skyr from the previous day's batch at a rate of 15 g/L of milk (optional), heated, cooled to 40°C, inoculated with an active liquid starter culture and rennet (6 mL/100 L) and fermented for ~5 h (pH 4.7). The fermentate is agitated vigorously, cooled to 18°C, fermented for 20 h (pH 4.2), heated to 68–70°C, cooled to 40°C, separated from the whey, partially cooled, packaged, and stored, A second fermentation stage is necessary for the yeast to grow and produce the required flavors in the product including alcohol (0.3–0.5%) and CO_2, and the heat treatment of the fermentate before the separation stage helps to inactivate the yeast (i.e., extend the shelf-life of the product) without drastically reducing the count of the lactic acid microflora.

Traditionally the whey is utilized in Iceland in food preparations, pickling of food, as an animal feed, and/or consumed as a beverage. However, UF of the whey from the nozzle separator to produce retentate of the same level of total solids as skyr could be utilized to increase the yield (see Fig. 67.3); to enhance the consumption of skyr, the product could be mixed with fruit flavors, sugar, and cream.

67.4.3 Fermented Milks with Mould and Lactic Starter Cultures

This Finish fermented milk product is similar to some Nordic fermented milks and is called viili, but the mesophilic lactic starter culture (e.g., a blend of *Lactococcus* and *Leuconostoc* ropy strains) is enriched with a mould, *G. candidum*. The fat in cows' milk is standardized to 2.5 g/100 g (the SNF is not fortified or homogenized), and the

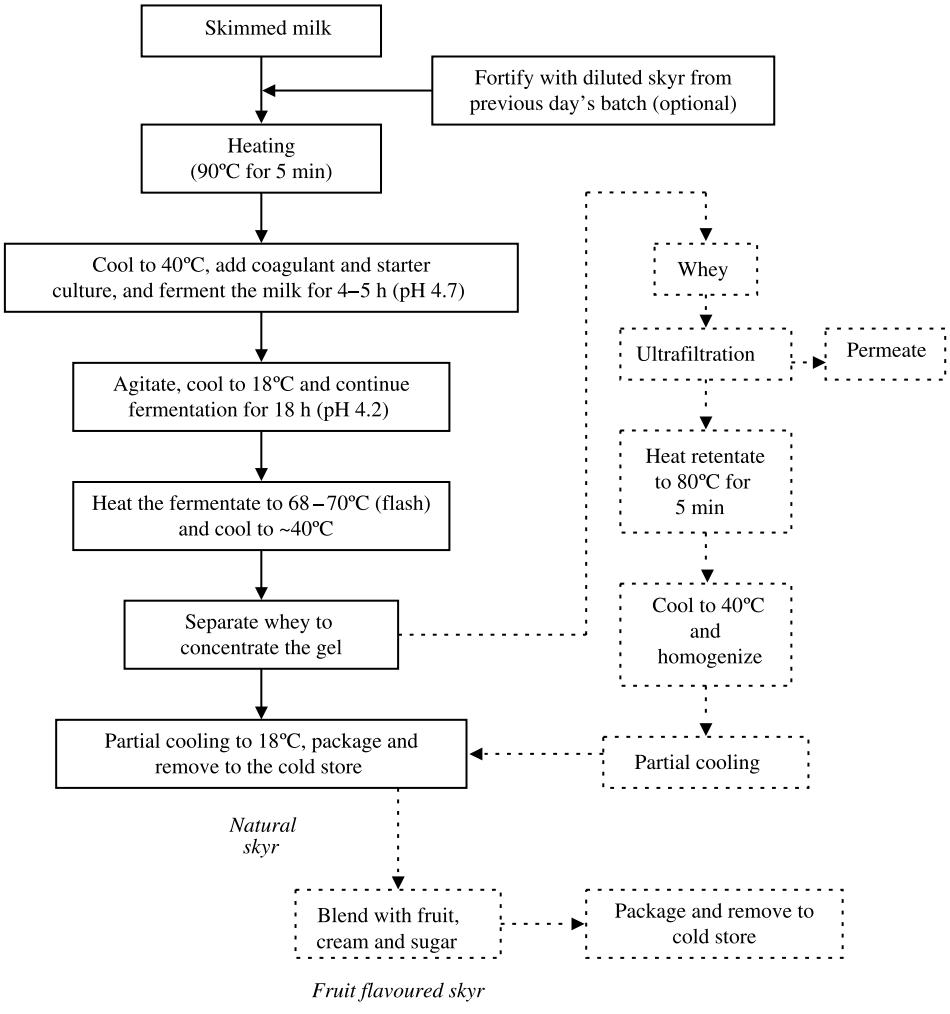

Figure 67.3 *Generalised flow chart for the production of skyr.*

milk is heated to 92–96°C for 4–5 min, cooled to 18–20°C, fermented to pH 4.4, blended with fruit (optional), packaged, and transferred to the cold store (Tamime and Marshall 1997; IDF 1988).

The product is mildly sour, aromatic in taste, and the texture is smooth, thick, and stretchy; the flavor is similar other buttermilk products, but it has a slight musty aroma that is attributed to *G. candidum*.

67.5 CONCLUSION AND FUTURE TRENDS

It is clear from the range of fermented milks on the market and their uptake by consumers that such products provide a major source of income for the dairy industry. For this success to continue, a degree of innovation will be needed. Evidence of "new products" can be found in relation to modified blends of fruit flavorings in standard

stirred yogurts, the higher solids and fat contents in, for example, Greek-style yogurts and the use of "new" cultures that enhance the organoleptic or alleged therapeutic properties of the products.

Indeed, the future for fermented milks looks extremely bright provided, of course, that manufacturers avoid two obvious dangers. One would be to allow onto the market alleged "health-promoting" products with low bacterial counts, or alternatively, high counts of an inappropriate organism(s) (Robinson and others 2002), while the other would involve a link being established between certain fermented milks and a food-borne infection like salmonellosis (Al-Haddad and Robinson 2003). As both these risks should be easy to control, there is no reason why the Western markets for fermented milks should not continue to expand.

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68

Fermented Milk in Asia

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68.1 Introduction 432
68.2 Background 432
68.3 Classification of Fermented Milk in Asia 432
68.3.1 Yogurt 432
68.4 Trends of Fermented Milks in Asia 433
68.4.1 Yogurt Types on the Basis of Texture Characteristics 434
68.4.2 Requirement for Fermentated Milk Production 434
68.4.2.1 Milk Quality 434
68.4.2.2 Quality and Functionality of Other Ingredients (Stabilizer, Texturizer) 435
68.4.2.3 Pasteurization of Milk 436
68.4.2.4 Homogenization of Milk 437
68.4.2.5 Types of Culture 437
68.4.2.6 The Fermentation Process for Bulk Production 440
68.4.2.7 Shelf-Life of Product 441
68.4.3 Process Detail of Product Type 441
68.4.3.1 Set Yogurt/Stirred Yogurt 441
68.4.3.2 Drinking Yogurt 442
68.4.3.3 Frozen Yogurt 443
68.4.3.4 Concentrated Yogurt 443
68.4.3.5 Kefir 443
68.4.3.6 Cultured Cream 444
68.4.3.7 Buttermilk 446

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

68.4.3.8 Fermented Buttermilk 446
68.4.3.9 Probiotic Cultured Milk Products 446
68.5 Conclusion and Some Remarks on Market's Trends 447
References 447

68.1 INTRODUCTION

Fermented milk has many variants, for example, yogurt, ymer, kefir, cultured buttermilk, filmjolk (Scandinavian sour milk), culture cream, and koumiss. It is prepared by the inoculation of milk products with a starter culture. Lactic acid bacteria is usually used for fermentation (e.g., yogurt) or a combination of this and yeast fermentation (e.g., kefir). A starter culture converts part of lactose in milk product to lactic acid, produces carbon-dioxide, acetic acid, diacetyl, acetadehyde, and several other substances. The microorganism used in kefir and koumiss production also produces ethyl alcohol to provide taste and aroma. The low pH of fermented milk inhibits the growth of putrefactive bacteria. That is why fermented milk can sustain the quality of product over its shelf-life better than most other milks.

68.2 BACKGROUND

Fermented milk including kefir and yogurt originated from the Middle-East in and around Turkey at slope of Mt. Elbrus in the Caucasus, and subsequently became popular in Eastern and Central Europe. By accident, microorganisms of various kinds were fermented in a pitcher of milk at the right temperature and became activated with symbiosis. In the 11th century the name of "Yogurt" becoming well-known and it metamorphosed to "Yogurt" from the 11th century onward. Yogurt culture is mostly thermophilic bacteria. It prefers high temperature at 40–45°C while kefir culture, a mesophile, prefers moderate temperature at 25–28°C. The name kefir is derived from "kef," a Turkish name which means pleasurable. Kefir is fermented from different types of lactic acid bacteria, and yeast which are capable of forming alcohol at approximately 0.8%.

68.3 CLASSIFICATION OF FERMENTED MILK IN ASIA

68.3.1 Yogurt

In Asia, "yogurt" is the most popular fermented milk, consumed by over approximately 1.8 billion kg (LE 2003). The consumption is highest in countries around the Mediteranian, Japan, Korea, and South-East Asia. Consumers in each area prefer different types of aroma, flavor, and texture. For instance in Thailand, Vietnam, Indonesia, and Malaysia, consumers prefer less viscous liquid product while in the mediteranian area they prefer higher viscous liquids. In Japan people prefer cultured drinking yogurt with probiotics dominating usage. Yogurt is classified according to the way of preparation as follows:

- Set yogurt is the yogurt which is incubated and cooled in final packages.
- Stirred yogurt is the yogurt which is fermented in large vats, structured, and then filled into the final packaging.
- Drinking yogurt is the yogurt in liquid form where it is produced similar to stirred yogurt, but difference in formula and texturizing process, yielding a much thinner product by adding fruit syrup before being packed.

Figure 68.1 *Product variants of stirred yogurt, set yogurt, and pasteurized drinking yogurt.*

- Frozen yogurt is the yogurt which is incubated in a tank and frozen like ice-cream.
- Concentrated yogurt is the yogurt which is incubated in tanks, concentrated and cooled before being packed. This type is sometimes also called "strain yogurt" or "labneh". This is extremely popular in the Middle-East, particularly Saudi Arabia. A more liquid variant is called "laban" or "salty yogurt."

68.4 TRENDS OF FERMENTED MILKS IN ASIA

In Asia, drinking milk is becoming less popular in more and more countries (Fig. 68.2), while fermented milk is becoming more popular. Drinking yogurt and stirred cup yogurt have been growing dramatically in Asia with the growth rate of between 4.8% to 5.5% from year 2001 to year 2002 (Fig. 68.1). Currently in the Thai and Vietnamese markets, the growth is double digit (11%), the fastest in South-East Asia. Preference on taste profile is very much regionally influenced. The consumers in each area prefer their own

Figure 68.2 *Product variants of UHT drinking yogurt in South East Asia.*

taste, flavor, and texture. The method of preparation, the types of culture, and the choice of ingredients play a major impact on the characteristics. Looking at new trend in Asia, we can see the following:

- More variations: switch from basic yogurts toward special value-added and luxury yogurts.
- Yogurt targeted at new consumption moments, meal replacers.
- Yogurt packaging becoming more and more aimed at convenience; fun and attractive.
- Health awareness leading to more "balanced diet". Product with nonfat and high calcium, low sugar and added vitamins and minerals and fiber and use of prebiotic and probiotic cultures. Product with neutraceutical benefits such as adding omega 3, omega 6, or DHA.
- Food scares fueling increased interest in GMO free, gelatin free, natural, and organic products.

68.4.1 Yogurt Types on the Basis of Texture Characteristics

Besides the classification of yogurt by the way of preparation or production, the texture characteristic and functionality can be a key area to categorize into additional groups.

- Yogurt mousse – aerated yogurt;
- Probiotic yogurt – these products distinguish themselves on the health benefits of the cultures they contain;
- Petit Suisse/Fromages frais – the product is fermented with cheese cultures; after whey separation the fresh curd is processed into a yogurt-like texture.

68.4.2 Requirement for Fermentated Milk Production

As yogurt is a regional product, the method of preparation, types of cultures, and choice of ingredients have a major impact on the final product. Each of these choices make the differentiations. Factors that play a crucial role in the production of good quality yogurt are as follow:

1. Milk quality
2. Quality and functionality of other ingredients (stabilizer, texturizer)
3. Pasteurization of milk
4. Homogenization of milk
5. Type of culture, format (ready set or direct vat set)
6. Fermentation process for mother culture and starter culture.

68.4.2.1 Milk Quality. The milk for yogurt preparation must have a low bacteria count, not contain any enzymes, bacteriophages, antibiotics, or any substance such as residue from CIP solution which may slow down the development of the yogurt culture.

- The milk ingredient may be made from fresh milk, ranging from skimmed milk to whole or recombined milk.
- In case of using recombined milk, it is important to ensure proper hydration. Correct hydration is also critical for protein stabilization.
- Nowadays, the application for other types of milk ingredient has become more popular such as whey protein and caseinate. These variants many times contribute to product stability, taste, and cost.

TABLE 68.1 Strength and Weakness of Milk Components Used in Yogurt Production.

Milk Ingredients	Strength	Weakness
Skimmed milk powder	Composition	Viscosity Protein content
Caseinate	Viscosity Protein content	Smoothness Dissolution
Total milk protein	Composition Protein content	Viscosity
Whey protein concentrate	Water binding Smoothness	Viscosity

- The purposes of milk ingredients are to supply substrate for culture growth, provide nutrients which include macronutrients (protein, fat, calories), and micronutrients (vitamins, minerals, nutraceuticals which are probiotic and prebiotic).
- Types of milk ingredient also produce desirable flavor compounds, provide desirable body and texture (Table 68.1).
- Casein can help developing of coagulum, whereas whey protein helps to form water binding, viscosity, and gelling. In terms of color, fat globules and casein can play an important role as well as riboflavin, which can provide yellow and greenish colors.
- Whey powder can improve flavor, texture, enrich nutritional value, reduce syneresis, and benefit neutraceutically. The overall functionality of milk ingredients can influence fermentation rate and quality of yogurt production.

68.4.2.2 Quality and Functionality of Other Ingredients (Stabilizer, Texturizer). Hydrocolloides (also known as stabilizer, texturizer) are used commonly for commercial purposes. They can bind water, increase viscosity, and help to prevent separation in yogurt. The types of stabilizers and dosages which should be added depending on desired product characteristic, is determined by the manufacturer. The primary aims of using texturizers are:

- Body firmness
- Smooth appearance
- Synersis prevention
- Gel formation
- Full taste, good mouth-feel
- Limit damage during structuring.

Common type of stabilizer in current use are:

- Pectin
- Gelatin
- Modified food starch
- Xanthan
- Guar
- Locust bean gum
- Polysaccharides produced by yogurt culture.

In natural yogurt no stabilizer is required because the firm and fine gel with high viscosity will come automatically. Stabilizers can be used in fruit yogurt and pasteurized yogurt to ensure stability.

Pectin is the commonly used stabilizer in the world. It is proved to be the most effective stabilizer. Pectin is made from the peel of various types of citrus fruits (apple, orange, and pomelo). Chemically pectin consists of partially esterified galacturonic bonds existing of macromolecules acid, which via its 1,4-glucosides (carboxyl groups are esterified with methoxyl group). Pectin is divided into two groups, high-ester pectin (HE) having a degree of esterification above 50% and low-ester pectin (LE) having a degree of esterification below 50%. High ester pectin is suitable for fermented milk.

How Does Pectin Work to Stabilize Protein? In natural milks with pH 6.6–6.7, caseins are found in stable micelles with a negative net charge. The micellar structure is broken down during fermentation at pH 5.2, the net charge of the casein is sufficiently low for curd formation to begin. Further fermentation causes a three-dimensional network of aggregated casein to build up, entrapping the surrounding serum phase. Maximum aggregation is obtained at pH 4.6, corresponding to the isoelectric point of caseins. By decreasing the pH further (e.g., pH 4), the net charge of casein becomes positive. High ester pectin can be bound to the protein surface as the blocks are negatively charged at pH 3.6–4.5, a typical level of acidified beverage. Bound to proteins, the long pectin molecule (molecular weight of up to 150,000) protect them from reaggregation through stearic stabilization.

The Method to Add Pectin. It depends on the process equipment and recipe.

There are three possibilities to add pectin:

- Prepare pectin solution: 0.05–5% pectin should be preblended with a minimum of five parts sugar. Strong agitation is necessary. A maximum 4–5% pectin solution.
- Pectin dispersion: 10% pectin and 90% sugar solution (65% SS saturated). The dispersion should be made at room temperature without subsequent heating to avoid partial pectin dissolution and increase in viscosity.
- Direct addition 10–20% pectin with 90% sugar for predrying. Strong agitation is necessary. Homogenization should take place in warm conditions to secure proper dissolution (upstream 60°C).

68.4.2.3 Pasteurization of Milk. The milk is pasteurized before being inoculated with the starter. This pasteurization results in improving the bacterial quality of milk by reducing level of psychotrophs and mesophiles. The objectives of pasteurizing milk are:

- Improving the properties of milk as the substrate for the bacteria culture.
- Ensuring that the coagulum of the finished yogurt will be formed.
- Reducing the risk of whey separation in the end product.

The optimum heat treatment is at temperature of 90–95°C for 5 min. This condition can denature 70–89% of the whey protein. In particular the beta-lactoglobulin, which is the principle whey protein, interacts with keppa casein, thereby helping to give the yogurt a stable "body."

68.4.2.4 Homogenization of Milk. Homogenization reduces the size of fat globules. It helps to improve the stability and consistency of fermented milk as it prevents "cream-off or fat separation" during the incubation period. Homogenization covers the fat globules with casein, a thin protein layer forming part of protein network. In case of extra proteins are being added to milk, homogenization also improves their distribution. It can be applied subsequently after pasteurization. The temperature for homogenization influences the viscosity. The higher the homogenization temperature, the more is the viscosity. Generally, it is recommended at 20–25 Mpa at 65–70°C. Homogenization is frequently utilized even for production of low fat cultured milk.

68.4.2.5 Types of Culture. Starter cultures for fermented milk products can be classified according to their preferred growth temperatures and their functionality as follows:

- A mesophillic bacterium is a bacterium which has optimum growth at 20–30°C.
- Thermophillic bacterium is a bacterium which has optimum growth at 40–45°C.
- Probiotic bacteria are a bacterium that colonies in the intestines and promote health growth (Tables 68.2 and 68.3).

The culture for fermented milk may be of single strain or multiple strain. Some *Strep. diacetylactis* bacteria are powerful acidifiers that can be used alone as acidifying cultures, but they are used primarily together with *Str. cremosis/lactis*. It is not possible to use a pure *Leuc. citrovorum* in milk which is conditional upon the availability of nutrients produced by *Str. lactis* or *Str. cremoris*. *Leuc. citrovorum* grows very slowly in milk in the absence of acid-producing bacteria, and cannot produce aroma substances in such conditions.

Forms of Starter Culture

- Liquid for propagation for mother culture which is nowadays very rare.
- Deep-frozen which is concentrated culture for preparation of bulk starter.
- Freeze-dried which is the concentrated cultures in powder form.
- Deep-frozen which is the superconcentrated cultures in readily soluble form, for direct inoculation of product.

Preservatives of Starters. The lower the temperature, the better the culture keeps. Modern forms of starter cultures can be stored in condition as recommended below in Table 68.4.

Stage of Propagation. Recently, cultures have generally been used as bulk starters as well as for direct use in production. Cultures in various stages propagation are known by the following names.

- Commercial culture, master culture – the original culture from the laboratory.
- Mother culture – the culture prepared from master culture.
- Intermediate culture – an intermediate step in the manufacture of large volume of bulk starter.
- Bulk starter – the starter used in production.

TABLE 68.2 Types of Culture Bacteria Used for Each Product.

Type of Bacteria		Product
	Mesophillic bacteria	
Old name	*Streprococcus cremoris*	Cheddar cheese
New name	*Lactococcus lactis ssp. Cremoris*	
Old name	*Streptococcus lactis*	Feta cheese
New name	*Lactococcus lactis ssp. lactis*	Continental cheese Mold-ripened cheese Cottage cheese Quarg
Old name	*Leuconostoc citrovorum*	Lactic butter
New name	*Leuconostoc mesenteroides ssp. cremoris*	
Old name	*Streptococcus diacetylacis*	Culture buttermilk
New name	Cit^{+}Lactococci	
	Thermophillic bacteria	
Old name	*Streptococcus thermophillus*	Yogurt
New name	*Streptococcus salivarius ssp.*	Emmental cheese
Old name	*Lactobacillus bugaricus*	Mozzarella
New name	*Lactobacillus delbrueckii ssp. bugaricus*	Yogurt
Old name	*Lactobacillus helveticus*	Grana cheese
New name	*Lactobacillus helveticus*	
	Probiotic bacteria	
Lactobacillus acidophilus		Culture DKY
Bifidobaterium bifidum		Culture DKY
Lactobacillus reuteri		Culture DKY

Preparation of Culture. The handling of the starter for production of fermented milk demands strict standards of hygiene. Very careful attention must be paid to the manufacturing technology and choice of process equipment. The risk of airborne infection by yeasts, molds, fungi, and bacteriophages must be absolutely minimized.

TABLE 68.3 Essential Data for Some Important Bacteria.

Bacteria	Optimum Growth Temp (°C)	Max. Salt Tolerance for Growth (%)	Acid Formation, Ferment (%)	Citric Acid Ferment
		Steptococci		
Str. Lactis	~30	4–6.5	0.8–1.0	−
Str. cremoris	25–30	4	0.8–1.0	−
Str. diacetylactis	~30	4–6.5	0.8–1.0	+
Str. thermophillus	40–45	2	0.8–1.0	−
Leuc. citrovorum	20–25	–	Small	+
		Lactobacillus		
Lb. helveticus	40–45	2	2.5–3.0	−
Lb. lactis	40–45	2	1.5–2.0	−
Lb. bulgaricus	40–50	2	1.5–2.0	−
Lb. acidophilus	35–40	–	1.5–2.0	−

TABLE 68.4 Storage Conditions and Shelf-Life of Cultures.

Type of Culture	Storage	Shelf-Life (months)
1. Freeze-dried super-concentrate (for direct incubation product)	Freezer below −18°C	Minimum 12
2. Deep-frozen	Freezer below −45°C	Minimum 12
3. Freeze-dried concentrated culture (for preparation of bulk starter)	Freezer below −18°C	Minimum 12
4. Deep-frozen concentrate culture (for preparation of bulk starter)	Freezer below −18°C	Minimum 12
5. Freeze-dried culture in powder for preparation of mother culture	Freezer below +5°C	Minimum 12

The preparation of culture comprises of the following stages:

- Heat treatment of medium
- Cooling to inoculation temperature
- Inoculation
- Incubation
- Cooling the finished culture
- Storage the culture.

HEAT TREATMENT OF MEDIUM. Skimmed milk is the medium most frequently used for starter production. Fresh milk from selected source which is proved to be free from antibiotic may be used as well. The medium can be modified by addition of growth factor such as Mn^{2+} (Manganese) to promote growth of *Leuc. citrovorum*. Phage-inhibiting media (PIM) offer an alternative for production of single-strain or multistrain starters. These media contain phosphates, citrates, or other chelating agents which make Ca^{2+} (calcium) insoluble. To remove Ca^{2+} from media protect lactic acid bacteria from being infected by phage as phages require Ca^{2+} for proliferation.

The media is heated to 90–95°C for 30–45 min. The heat treatment improves the properties of the medium through destruction of bacteriophages, elimination of inhibitor substances, some decomposition of protein, expulsion of dissolved oxygen, and destruction of original living microorganisms.

COOLING TO INOCULATION TEMPERATURE. The cooling temperature in order to inoculate the culture depends on type of culture (mesophillic bacteria or thermophillic bacteria) which may be recommended by supplier. Typical incubation temperature of mesophillic bacteria is 20–30°C and of thermophillic is 42–45°C.

INOCULATION. The amount of starter used, the propagation temperature and the fermenting time should be kept constant. The amount of starter used can affect the relative proportions of different bacteria which produce lactic acid and aroma substances. The optimum dosage which generally recommended is 2–3%.

INCUBATION. The incubation time can vary from 3 to 20 h depends on dosage of inoculum. During incubation period the bacteria multiply rapidly and ferment lactose to lactic acid, some of bacteria provide aroma substance such as diacetyl, acetic acid and propionic

acids, ketone, and aldehyes of various kinds, alcohols, esters, and fatty acids as well as carbon-dioxide.

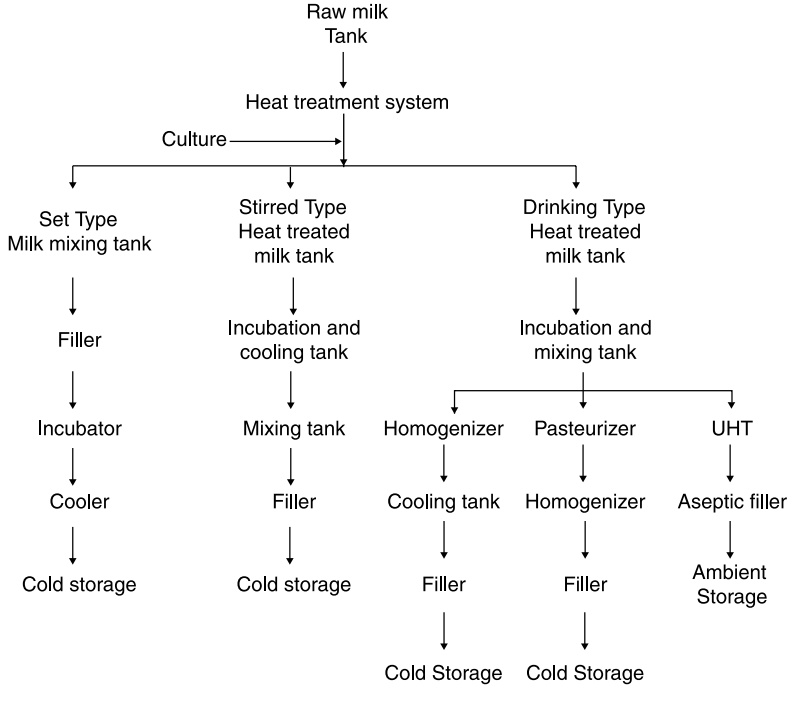
The culture which are commonly used for fermented milk in Asia are mixed culture of *Str. thermophillus* and *Lb. buegaricus*, which coexists in symbiosis and together produces the desired characteristics of the yogurt, such as pH, flavor, and aroma. Acetaldehyde is recognized as major flavor component in yogurt. Formation of acetaldehyde does not become evident until a certain level of acidification at pH 5.0. It reaches the maximum at pH 4.2 and stabilizes at pH 4.0. Most yogurts have a ratio between cocci to bacilli of between 1 : 1 to 2 : 1. The factor affecting the ratio of cocci to bacilli is the incubation temperature. At 40°C the ratio is about 4 : 1 while 45°C is 1 : 2. The optimum temperature for inoculation is thus 43°C to achieve cocci to bacilli ratio of 1 : 1, with the rate of inoculum of 2.5–3% and an incubation time of 3–4 h.

In addition, some strain of bacteria produce exopolysacharides (EPS) which have a notable influence on the structure of yogurt. The type of EPS and the fermentation period when the culture starts producing EPS both have impact on the final product. In this case, the low fermentation temperature (30–35°C) usually gives smooth and viscous yogurt. When fermenting with the same culture, it gives at temperature 40–45°C. One of the reasons is that at lower temperatures generally more EPS is produced.

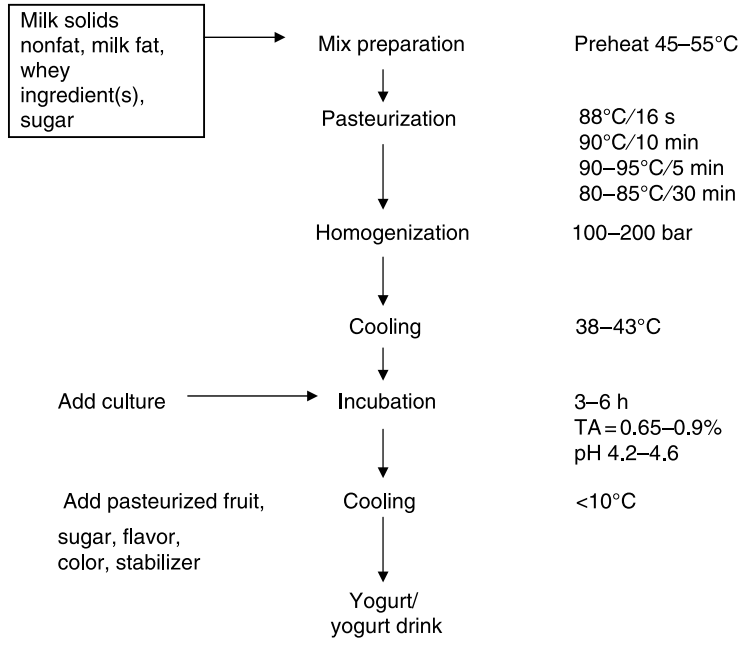
COOLING THE CULTURE. Cooling temperature at 10–12°C is common practice when culture is intended to be used within 6 h.

68.4.2.6 The Fermentation Process for Bulk Production

General Process Flow



Flowchart 1 *Process flowchart to compare different types of fermented milk in Asia.*



Flowchart 2 *Processing flowchart to basic production.*

68.4.2.7 Shelf-Life of Product. The Shelf-life of a pasteurized DKY product is approximately 1 month storage and distribution when the temperature does not exceed 7°C. In UHT drinking yogurt, shelf-life can be 6–10 months depending on the UHT process. Most spoilage problems are due to yeasts and molds which can become contaminated from starter, sugar syrup, fruit preparation, packaging machinery, and so on. Cleaning and sanitation minimize problems of spoilage and can extend shelf-life further.

68.4.3 Process Detail of Product Type

68.4.3.1 Set Yogurt/Stirred Yogurt. In order to reduce installation costs, it is possible to use the same plant for production of both stirred and set yogurt. The pretreatment of the milk intended for either product is identical up to cooling down of incubation temperature. The starter is metered into the stream of milk as it is pumped from an intermediate storage tank to the filling machine.

Flavoring/Packaging. Flavoring can be continuously metered into the milk stream prior to the filling machine. If fruit or additives with particles should be added, these have to be dosed into the packages or cups first before they are filled with fermented milk.

An Alternative Production System. In production of set yogurt, the milk is pretreated in the same way as for stirred yogurt, then cooled to a temperature of less than 10°C, preferably to 5°C, and pumped into one, two or more tanks. Following inoculation and thorough stirring, the milk is ready to be heated in-line to incubation temperature before being packed in containers. Bulk starter culture can also be added in-line prior to heating to incubation temperature.

Incubation and Cooling. Following packaging, the packages after crating and palletizing, are being trucked to the final cold store (an incubation room able to accommodate a large number of filled pallets). After adequate incubation the pallets are trucked to a conveyor passing through the cooling sections enclosed in a tunnel.

INCUBATION. The filled packages/containers are placed in crates of open design and at a certain distance from each other so that the circulating warm/cold air in the incubation and cooling room or chamber can reach every individual container. The crates are normally stacked on pallets, which are then trucked into the incubation room. This ensures uniform quality, provided that the temperature is accurately controlled.

COOLING. When the empirically determined optimum pH (typically 4.5) is reached, it is time to start cooling. The normal target temperature is 18–20°C; it is important to stop further growth quickly, which means that a temperature of about 35°C should be reached within 30 min, and 18–20°C after another 30–40 min. Final cooling, normally down to 5°C, takes place in the chill store, where the products are held to await distribution. The pallets (crates) are stationary during incubation. They are placed in the incubation room/chamber in such a way as to facilitate first in/first out handling. In a typical incubation period of 3–3.5 h, it is very important that the product is not exposed to any mechanical disturbance during the last 2–2.5 h, when it is most sensitive to the risk of whey separation.

The cooling capacity should be adequate to achieve the abovementioned temperature program. As a guide, it may be mentioned that the total cooling time is about 65–70 min for small packages (0.175–0.2 kg sizes) and about 80–90 min for large packages (0.5 kg size). Eventually, regardless of the type of incubation/cooling chamber, the set yogurt is cooled to about 5°C in the chill store.

68.4.3.2 Drinking Yogurt. A low-viscosity drinkable yogurt, normally with a low fat content, is popular in many countries such as Thailand, Vietnam, Malaysia, Japan, China, and so on. The yogurt intended for production of drinking yogurt is produced in the ordinary way as described above. Following stirring up and cooling to about 18–20°C, the yogurt is transferred to the buffer tank prior to the process alternatives. Stabilizers and flavors are mixed with the yogurt in the tank. The yogurt mix can then be treated in different ways, depending on the required shelf-life of the product.

Long-life Drinking Yogurt. Because of the tendency towards larger and more centralized production units, the markets in Asia are becoming geographically larger and transport distances are longer. Therefore, it is necessary to extend the shelf-life of the product beyond normal. In some countries it is difficult to maintain the integrity of the cooling chain. Therefore, there is demand for a sterilized yogurt that can be stored at room temperature.

The shelf-life of cultured milk products can be extended in two ways:

- Production and packing under aseptic conditions such as in bottles;
- UHT heat treatment of the finished product, either immediately before packing or in the UHT package.

68.4.3.3 Frozen Yogurt. Frozen yogurt can be manufactured in two ways. Either yogurt is mixed with an ice cream mix or a yogurt mix is fermented, before further processing. Frozen yogurt can be divided into soft-served and hard-frozen types. The mix intended for soft-served yogurt differs somewhat from that of the hard frozen type. Typical recipes are as follows.

Hard-Frozen Yogurt. As in the case of conventional ice cream, the yogurt is prefrozen and whipped in a continuous ice-cream freezer. Whipping takes place in a nitrogenous atmosphere to avoid oxidation problems during subsequent storage. The frozen yogurt leaves the freezer at $-8^{\circ}C$, which is somewhat lower than the temperature of conventional ice cream. This gives it an optimum viscosity that suits most filling machines.

Liquid fruit flavoring or sugar can be added in the freezer. Frozen yogurt with different flavors can be produced in parallel freezers from a common yogurt mix. After freezing, the frozen yogurt is packed into cones or cups or family size packs in the same way as conventional ice cream. The packs then go into a hardening tunnel, where the temperature is reduced to $-25^{\circ}C$.

Frozen yogurt bars can be frozen continuously in a regular ice cream bar freezer. Since the yogurt is frozen directly to $-25^{\circ}C$, it can be transported to the cold store immediately after packaging.

Shelf-Life and Distribution. *Hard-frozen* yogurt which is whipped with nitrogen can be kept in cold storage for 2–3 months without any adverse effects on its flavor or texture. Distribution requires an unbroken cold chain right up to the instant of consumption. In the case of the mix for *soft-served yogurt* (not subjected to UHT treatment), a maximum storage temperature of $+6^{\circ}C$ is recommended. This mix has a storage life of a couple of weeks. Soft-served yogurt is consumed immediately after freezing.

68.4.3.4 Concentrated Yogurt. In concentrated yogurt the dry matter (DM) of the product is increased after fermentation. Whey is drained off from the coagulum. The manufacturing principles are identical with the manufacturing of quarg. The only difference is the type of cultures used. Concentrated yogurt is known under names such as "strained" type yogurt and Labneh.

68.4.3.5 Kefir. Kefir is one of the oldest cultured milk products. It originates from the Russian Caucasus region (bordering Turkey). The raw material is milk from goats, sheep, or cows. Kefir is produced in many countries, although the largest quantity – an annual total of about 5 L per capita – is consumed in Russia. Kefir is viscous and homogenous, and has a shiny surface. The taste is fresh and acid, with a slight flavor of yeast. The pH of the product is usually 4.3–4.4. A special culture, known as kefir grain (Fig. 68.3), is used for the production of kefir. The grains consist of proteins, polysaccharides, and a mixture of several types of microorganisms, such as yeasts and aroma, and lactic-acid forming bacteria. The yeasts represent about 5–10% of the total microflora (Fig. 68.5).

The kefir grains are yellowish in color and about the size of a cauliflower floret, that is, about 15–20 mm in diameter. The shape of the grains is irregular. They are insoluble in water and in most solvents. When steeped in milk, the grains swell and become white. During the fermentation process, the lactic-acid bacteria produce lactic acid, whereas the lactose-fermenting yeast cells produce alcohol and carbon-dioxide. Some breakdown of protein also takes place in the yeast metabolism, from which kefir derives its special

yeast aroma. The contents of lactic acid, alcohol, and carbon-dioxide are controlled by the incubation temperature during production.

68.4.3.6 Cultured Cream. Cultured cream has been used for years in some countries. It forms the basis of many dishes in the same manner as yogurt. Cultured cream can have a fat content of 10–12% or 20–30%. The starter culture contains are *Str. lactis* and *Str. cremoris*, whereas *Str. diacetylactis* (D and DL cultures) and *Leuc. citrovorum* (DL and L cultures) bacteria are used for the aroma (Fig. 68.4).

Cultured cream is bright, has a uniform structure, and is relatively viscous. The taste is mild and slightly acid. Cultured cream has a limited shelf life. Strict hygiene is important to product quality.

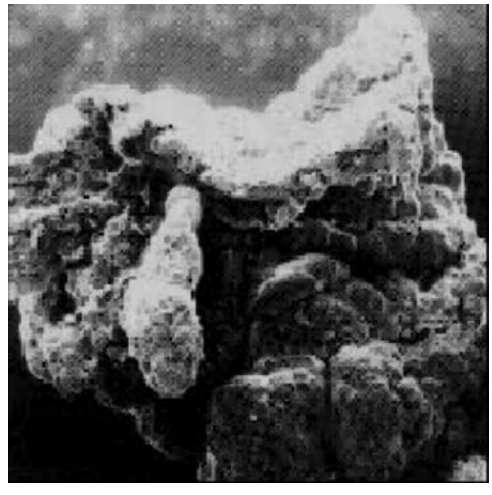


Figure 68.3 *Kefir grain.*

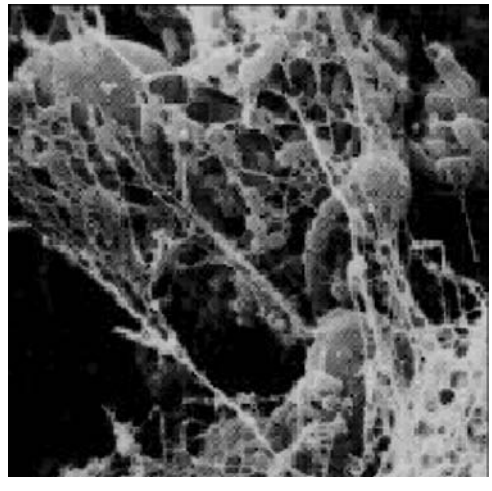


Figure 68.4 *The microorganisms in cultured products often live in symbiosis with each other.*

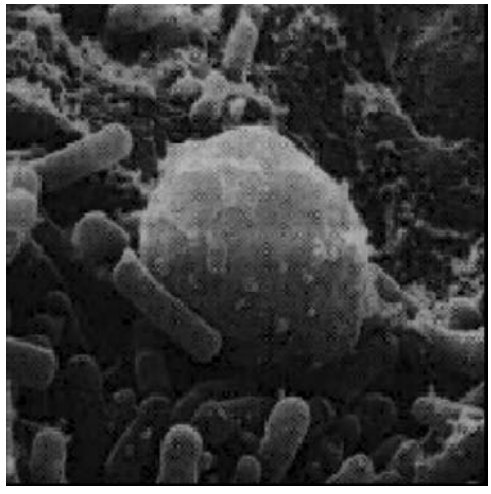


Figure 68.5 *Yeast and lactic acid at the surface of a kefir grain, seen through an electron photomicroscope.*

Yeast and molds can develop in packages which are not airtight. These microorganisms occur mainly on the surface of the cultured cream. In the event of extended storage the lactic-acid bacteria enzymes, which break down β-lactoglobulin, become active and the cultured cream goes bitter.

The cultured cream also loses its flavor because carbon-dioxide and other aromatic substances diffuse through the packages.

Production. The process line for production of cultured cream includes equipment for standardization of the fat content, homogenization, and heat treatment of the cream, and also inoculation and packing.

Homogenization. The cream is homogenized. For cream with 10–12% fat the homogenization pressure is normally 15–20 MPa (150–200 bar) at 60–70°C. Up to a certain point, an increase in homogenization temperature improves the consistency.

For cream with 20–30% fat the homogenization pressure should be lower, 10–12 MPa (100–120 bar), as there is not enough protein (casein) to form membranes on the enlarged total fat surface.

Heat Treatment. The homogenized cream is normally heat treated for 5 min at 90°C. Other time/temperature combinations can be used if the homogenization technique is carefully matched to the heat treatment.

Inoculation and Packing. The pretreated cream is cooled to an inoculation temperature of 18–21°C. One to two percent of bulk starter culture is then added. Inoculation can take place in a tank or in the packages. The fermentation time is 18–20 h. When fermentation is completed, the cultured cream is cooled quickly to prevent any further pH reduction. The viscosity of the fermented cream may be very high, and it may therefore be difficult to pack.

In spite of precautions, the mechanical treatment to which the cultured cream is subjected during stirring, pumping, and packing also causes a slight deterioration in the consistency of the product – it will become thinner.

Cultured cream is bright, has a uniform structure and is relatively viscous. The taste should be mild and slightly acid. The cream is sometimes inoculated, packaged, and fermented in the packages to avoid mechanical treatment. After inoculation of the cream and subsequent packing, the product is stored at 20°C until the acidity of the fat-free phase is about 85°C, which takes about 16–18 h. The packages are then carefully transferred to the chilled store, where they are kept for at least 24 h at a temperature of about 6°C before distribution. Cultured cream is often used in cooking.

68.4.3.7 Buttermilk. Buttermilk is a by-product of butter production from sweet or fermented cream. The fat content is about 0.5%, and it contains a lot of membrane material including lecithin. The shelf life is short, as the taste of the buttermilk changes fairly quickly because of oxidation of the membrane material content. Whey separation is common in buttermilk from fermented cream, and product defects are therefore difficult to prevent. Buttermilk is growing in popularity. Consumers have a preference for a silky taste and more yellow color.

68.4.3.8 Fermented Buttermilk. Fermented buttermilk is manufactured on many markets in order to overcome problems such as off-flavors and short shelf-life. The raw material can be sweet buttermilk from the manufacture of butter based on sweet cream, skim milk, or low-fat milk.

In all cases the raw material is heat treated at 90–95°C for about 5 min before being cooled to inoculation temperature. Ordinary lactic-acid bacteria are most commonly used. In some cases, when the raw material is skim milk or low fat milk, grains of butter are also added to the product to make it look more like buttermilk.

68.4.3.9 Probiotic Cultured Milk Products. Probiotic culture milk is a "functional food or neutaceutical product." The name is applied to foods with near-medicinal properties that promote health. "Food for special health use" is another term for the same thing. Japan is at present the leading country for "functional food" and has a great preventive program of schemes to lower the costs of medical treatment. Lactic acid bacteria have been used since time immemorial to ferment foods. The special strains of bacteria normally used in production of yogurt, as well as other types such as *Lactobacillus* (*L.*) *acidophilus*, *L. reuteri* (a relative newcomer), Bifido-bacteria and certain species of *Lactococcus lactis*, are among those that have been found of interest for production of functional foods.

How a Lactic Acid Bacterium Function in the Intestine. The four characteristics that are of primary importance are:

- Ability to colonize and survive
- Adhesive capacity
- Ability to aggregate
- Antagonistic effects.

L. acidophilus and Bifido-bacteria are important members of the human intestinal flora. The former normally predominates in the small intestine and the latter in the large intestine. Production of these important bacteria is reduced in some people as a result of medication, stress, or old age. In many people, reduced production of intestinal bacteria can cause symptoms such as swelling, indigestion, and pronounced illness.

Consumption of live *L. acidophilus* and Bifido-bacteria in milk products is an ideal way to restore the balance of the intestinal flora. Apart from the possible prevention and relief of diarrhea, literature indicates that *L. acidophilus* and Bifido-bacteria may help to:

- Reduce the cholesterol level in the blood
- Relieve lactose mal absorption (lactose intolerance)
- Strengthen the immune system
- Reduce the risk of stomach cancer.

The production of probiotic bacteria is similar to drinking-yogurt. However, the preferred temperature for growth is around 32–38°C and it needs longer fermentation time (60–72 h).

68.5 CONCLUSION AND SOME REMARKS ON MARKET'S TRENDS

The fermented milk landscape is very broad and colorful. The functionalities are growing. What started centuries ago purely as a basic form of nutrition is today moving towards fulfilling needs beyond basics. Enhanced nutritional needs as well as emotional needs are the routes being pursued by manufacturers who are looking for new categories, niches, and points of differentiation. What we can safely say about the future is that the further development of this categories will continue. The reasons are not only commercial but also social and cultural. Companies across Asia today are investing significant amounts of resources to bring new innovations to the market place. Branding, positioning, differentiation on functional and emotional benefits, quality, taste, packaging, robustness, survivality in the supply chain are the key issues for the future.

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69

Goat Milk, Its Products and Nutrition

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69.1 Introduction 450
69.2 Production of Goat Milk 451
69.2.1 Goat Milk Production in the World Compared to Other Dairy Species 451
69.2.2 Breeds and Milk Production 452
69.2.3 Economics of Improved Goat Milk Production 454
69.2.4 Uniqueness of Goat Milk 458
69.3 Chemical Composition of Goat Milk 458
69.3.1 General Compositional Characteristics of Goat Milk 458
69.3.2 Factors Affecting Composition and Physicochemical Properties of Goat Milk 459
69.3.2.1 Diet 459
69.3.2.2 Breed 460
69.3.2.3 Stage of Lactation 460
69.3.2.4 Season 461
69.4 Chemical Characteristics of Goat Milk Compared to Other Species Milk 461
69.4.1 Lipids 461
69.4.1.1 General Composition of Goat Milk Lipids 461
69.4.1.2 Fatty Acid Composition of Caprine Milk 463
69.4.1.3 Free Fatty Acids in Caprine Milk 463

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

69.4.2 Proteins 464
69.4.2.1 Major Proteins and Caseins in Goat Milk 464
69.4.2.2 Whey Proteins 465
69.4.2.3 Amino Acid Composition 465
69.4.2.4 Nonprotein Nitrogen and Other Nitrogen Moieties of Goat Milk 465
69.4.3 Minor Proteins, Enzymes, and Other Constituents 467
69.4.4 Carbohydrates 468
69.4.5 Minerals 468
69.4.6 Vitamins 469
69.5 Protein Polymorphism and Rennetability of Goat Milk 470
69.5.1 Protein Polymorphism in Goat Milk 470
69.5.2 Variants and Alleles at the Milk Protein Loci 471
69.5.3 Genetic Typing 471
69.5.4 Effect of Milk Protein Polymorphism on Renneting Properties 472
69.6 Physicochemical Properties and Processing Characteristics of Goat Milk 472
69.6.1 Physicochemical Properties and Processing Variables 472
69.6.2 Heat Stability of Goat Milk 473
69.7 Goat Milk Products and Their Manufacturing Technology 474
69.7.1 Beverage Milk 474
69.7.2 Goat Milk Cheeses 476
69.7.3 Goat Milk Yogurt 477
69.7.4 Other Goat Milk Products 478
69.7.4.1 Other Fermented Goat Milk Products 478
69.7.4.2 Evaporated and Powdered Goat Milk Products 479
69.7.4.3 Frozen Goat Milk Products 479
69.8 Advantages of Goat Milk in Human Nutrition and Health 479
References 481

69.1 INTRODUCTION

Although dairy cows produce the greatest amount of world milk supply mostly from developed countries, more people drink the milk of goats than milk of any other single species on a worldwide basis (Haenlein and Caccese 1984; Park 1990, 1994b). The FAO yearbook shows that dairy goat and dairy sheep farming is very important for the national economy of many countries, especially in the Mediterranean region. Dairy goat farming is a vital sector of agricultural businesses in developed countries of the Mediterranean region such as France, Italy, Spain, and Greece. This fact may prove that goat and sheep dairying is not necessarily synonymous with underdevelopment and poverty (Haenlein 2002).

However, production of goat milk is very important in countries of the underdeveloped world, where it provides basic nutrition and subsistence to the rural people, which are the majority of their populations. Production of goat milk and its products of cheeses and yogurt is also a valued part of the total dairy industry in developed countries, where it provides diversity to sophisticated consumer tastes, and supports people with medical afflictions, such as allergies and gastrointestinal disorders, who need alternative dairy products (Haenlein 1986, 1996, 1997; Park 1992b, 1994b). Thus, goat milk serves in a general way

TABLE 69.1 Average Daily Supply of Calcium and Protein Per Person (FAO 1995) in Relation to Recommended Dietary Allowances (RDA, 800 mg for Calcium and 60 g for Animal Protein; NRC 1964).

		From Animal Sources	
	Total Supply (mg)	(mg)	(% of RDA)
Calcium			
Africa	384	132	16
Asia	329	125	16
Europe	896	684	86
N + C America	832	569	71
S America	490	317	40
Protein			
Africa	56	12	20
Asia	64	16	27
Europe	101	58	97
N + C America	97	56	93
S America	67	31	52

three types of markets around the world, such as (1) home consumption, (2) specialty gourmet interests, and (3) medical needs.

Home consumption of goat milk is important in the prevention of undernutrition and malnutrition, since milk is the superior source of calcium and protein, and to millions of rural poor people cow milk is not available or not affordable. A daily minimum supply of 800 mg calcium per person is widely recommended, as is also a minimum of 60 g protein from animal sources (NRC 1964). Table 69.1 shows how far below these recommendations the average supply of calcium and protein is estimated to be especially in Africa and Asia.

69.2 PRODUCTION OF GOAT MILK

69.2.1 Goat Milk Production in the World Compared to Other Dairy Species

The changes in trends of world human population and milk production during the last 20 years are shown in Table 69.2. While the world population of people increased by 39% from 4.4 to 6.1 billion during the last 20 years, the milk supply tonnage from cows increased by 20%, from buffaloes by 168%, from sheep it did not change, but from goats it is estimated to have risen from 7 to 12 million MT or 71% (FAO 1986, 2003). At the same time, dairy cow numbers increased by 6%, buffaloes by 37%, but goat numbers jumped by 63% from 457 to 743 million head during these last 20 years. Thus, the worldwide dynamics of goat numbers and goat milk production tonnage are impressive. Likewise, the upward trends in goat population numbers and in goat milk production tonnage, especially in Africa and Asia, reflect the recognition of people for the significant contribution, that goat milk and its products make to people's nutrition and their social well-being.

TABLE 69.2 Trends of Population Numbers and Milk Production During the Last 20 Years.

	1981	2002	Change, %
	Population Numbers, Million		
People/world	4450	6134	39
Dairy cows, world	213	225	6
Buffaloes, world	122	167	37
Sheep, world	1090	1034	−5
Goats, world	457	743	63
Africa	150	217	45
Asia	264	470	78
N + C America	13	14	8
S America	19	23	21
Europe	12	18	50
	Milk Production, Million MT		
Dairy cows, world	417	502	20
Buffaloes, world	28	75	168
Sheep, world	8	8	0
Goats, world	7	12	71
Africa	1.7	2.7	59
Asia	3.5	6.3	80
N + C America	0.3	0.3	0
S America	0.1	0.2	100
Europe	1.6	2.5	56

Source: FAO 1986, 2003.

69.2.2 Breeds and Milk Production

Several hundred different breeds of goats are officially recognized worldwide, some specifically for Mohair or Cashmere fiber production, the majority for dual purpose production of meat and milk, and a few selected single purpose breeds for meat or milk production. They have been described and illustrated in detail in recent publications (Muňoz and Tejon 1980; Haenlein 1981; Mason 1981; Acharya 1982; Rubino 1990; Gall 1996; Devendra and Haenlein 2003). Goat breeds have also been categorized morphologically as well as by level of milk production into high, medium, and low milk-producing breeds, but feeding, climate, and management systems are often the major factors determining the level of milk production by different goats and by breeds (Flamant and Morand-Fehr 1982). On the other hand, superior genetic selection for high milk yield per day and for long lactations (up to 10 months) has established several breeds as so-called "improver" breeds for developing countries. These are especially the Swiss breeds: Saanen, Alpine, Toggenburg, Oberhasli; and also the American La Mancha and Anglo-Nubian, the Mediterranean Maltese, Canaria, Malagueňa, Murciana-Granadina, Damascus, and the Indian Jamunapari. Table 69.3 shows the levels of top milk production records achieved through superior genetic selection within breed and through excellent management of the individual does of some of the dairy goat breeds. These records also demonstrate, that improvement in milk production tonnage is possible without increasing population numbers, which is environmentally important, where increased herd numbers can lead to overgrazing pressure on the natural pasture regrowth, that has caused much concern in the past in some regions with dangerous desertification problems and public antagonism to goats (Haenlein 2001). The average

TABLE 69.3 Leading 10-Months Milk, Fat, and Protein Production Records of Officially Tested Individual Does of U.S. Dairy Goat Breeds (ADGA 2003), and Official U.S. Breed Average Production Records (Thomas and Haenlein 2004).

	Age, Years (Number of Does)	Milk, Lactation Record, kg	Fat		Protein		Year of Record (Number of Herds)
			kg	%	kg	%	
		Individual Goats, Leading Records					
Alpine	7	2916	140	4.8	93	3.2	1982
LaMancha	6	2454	81	3.3	74	3.0	1991
Nubian	4	2700	138	5.1	98	3.6	1996
Oberhasli	3	2120	106	5.0	61	2.9	1997
Saanen	4	2987	76	2.6	74	2.5	1997
Toggenburg	3	3620	142	3.9	109	3.0	1997
		Breed Averages					
Alpine	(1682)	947	33	3.5	28	2.9	(65)
LaMancha	(553)	767	29	3.7	24	3.1	(38)
Nubian	(1324)	680	31	4.6	24	3.6	(96)
Oberhasli	(190)	669	25	3.7	20	2.9	(19)
Saanen	(684)	873	31	3.5	26	2.9	(32)
Toggenburg	(341)	842	28	3.3	24	2.9	(22)
Crossbreeds	(5588)	820	30	3.7	25	3.0	(41)
All	(10,362)	821	30	3.7	25	3.0	(385)

production records for each breed shown in Table 69.3 are also much above those reported for other single purpose and even more for dual purpose goat breeds.

Different from the production of mohair from the U.S. Angora industry, statistics of the U.S. dairy goat industry concerning population numbers and milk marketing are not readily available, because no central organization is keeping track of this other than the voluntary registration of pedigreed goats by a minority of U.S. goat keepers (Haenlein 2001). Estimates of the total U.S. dairy goat population have been reported to be about 1 million head with an estimated 50,000 MT commercial goat milk/year (Haenlein 1986; Thomas and Haenlein 2004). The state of Wisconsin processed currently around 7000 MT/year. About 12,000 MT of goat milk are manufactured into powder in California annually, and an ever growing number of presently at least 100 farmstead cheese makers produce an increasing number of artisanal goat cheeses, soft and aged, for the rising U.S. cheese market. Consumption of cheeses in the United States, especially specialty cheeses from goat and sheep milk, has increased from 8 to 14 kg/head/year during the last 20 years.

Approximately 300 U.S. goat breeders have a license for marketing goat milk. It has been estimated, that a potential but unfulfilled goat milk market of 12,000 MT/year exists in the United States just for "biomedical" reasons of people afflicted with allergies and gastrointestinal disorders, calculated from a conservative average need of 1 L goat milk/1000 persons/week (Haenlein 1986). However, 22 U.S. states have presently no licensed goat dairy farm/processor, and three-quarters of the larger U.S. cities have no fluid goat milk available in stores. Urban U.S. consumers are thus confronted with the choice of mail ordering frozen goat milk from another state or buying goat milk powder from a Health Food store or keeping their own goat in the backyard of their home. Compare this situation with the dairy goat industry in Taiwan, where UHT goat milk is delivered daily to the doorsteps of consumers by a well organized cooperative (Morgan 1996). French goat milk is also exported widely into other countries, where potential markets with a deficit supply exist. It has been reported, that, for example, in Brazil 4.5 million liters of goat milk are marketed annually generating $12 million, but that a potential market of 12 million liters exists despite the higher retail price compared to cow milk (Vidal and others 2004).

69.2.3 Economics of Improved Goat Milk Production

As seen in Table 69.3, the improvement of goat milk production is not only possible genetically, that is, through selection within breed or crossbreeding between breeds, but is also achievable through superior management, that is, disease control and supplementary feeding. Searching the world research literature, it becomes clear that much more is known about the benefits of superior management, than is practiced today, and that is lacking in extension education of the many people in the "hinterland," who keep and depend on goats for their livelihood. It has been proven repeatedly (Table 69.4), that goats' low productivity in many regions is mostly due to underfeeding in energy and protein sources, which can be corrected by supplementary feeding in addition to the pasturing, and which is not costly but profitable contrary to widespread popular opinions (Sands and McDowell 1978; Yazman 1984; Devendra 1987; Morand-Fehr 1990; Sagar and Ahuja 1993; Hatziminaoglou and others 1995; Haenlein and Abdellatif 2004). The reason is the milk price : feed price ratio. As long as the supplement feed price/kg to the farm is less than half of the price of milk/kg from the farm, it is profitable to buy

TABLE 69.4 Economics of Improved Goat Milk Production.

Influence of feeding level (Sands and McDowell 1978)								
Feeding multiples of maintenance requirements					Expected milk yield/lactation, kg			
1.15–1.50–1.80–2.45					50–198–305–535			
Influence of plane of nutrition in India (Devendra 1987)								
Low plane of nutrition before and after kidding:					49 kg Lactation milk yield			
Medium plane before kidding and high plane during lactation:					109 kg Lactation milk yield			
High plane before and after kidding:					152 kg Lactation milk yield			
Economics of goat herds in eight districts of Rajasthan, India (Sagar and Ahuja 1993)								
Milk production, kg/doe/year:	113	135	178	254	375	434	576	595
Expenditures, Rupees/doe/year:	29	23	102	48	164	80	160	174
Net income, Rupees/doe/year:	84	112	76	206	211	354	416	420
Economics of goat husbandry in Greece (Hatziminaoglou and others 1995)								
Gross income/doe/year	$ 66 – Extensive Feeding				$ 135 – Intensive Feeding			
Expenses/doe/year	$ 58 – Extensive Feeding				$ 111 – Intensive Feeding			
Net income/doe/year	$ 8 – Extensive Feeding				$ 24 – Intensive Feeding			
Average net income in four quartiles of 120 U.S. goat herds, official records (Haenlein and Abdellatif 2004)								
Milk yield, kg/doe/lactation:				514	701	820	1050	
Cost of concentrate feed/doe/year, $:				83	91	98	109	
Income over feed cost/doe/year, $:				183	262	302	395	

concentrate feeds or grains to supplement the basic forage ration of the goats. This ratio of $<0.5:1$ also rules an improved feeding system, where 1 kg supplement should be fed for each 2–3 kg milk/doe/day, depending on fluctuations of price, composition in protein and energy, and body condition of the doe.

Obviously, the economics of goat milk production is not only very much influenced by the price of feeds, which generally comprise 50% of the cost of milk production, but also by the price of goat milk payable to the producer or by the market price paid by consumers (Haenlein 1997, 1998). If the goat milk producer can manage direct sales of his/her milk, cheese and/or yogurt to consumers, as seen along highways, for example, in Venezuela, Holland, France, Germany, or through mail orders, for example, in the United States, then a best possible price/kg goat milk is obtainable. Many goat milk producers are not in that fortunate position, but live in remote areas, and what they want to sell has to go through a wholesale dealer and distributor, who often takes as much a share or more of the final retail price as the producer is paid. Thus, the producer has to calculate what his/her production costs per kg goat milk are, in order to survive economically (Ace 1978; Yazman 1984; Olorunnipa and others 1990; Gebremedhin and Gebrelul 1991).

Economic theory states, that the cost of production per unit of product is decreased by increasing the numbers of products per production unit. This applies also to goat milk production. As seen in Table 69.4, net income increases with higher level of milk production. Furthermore, the cost/kg goat milk decreases with higher levels of production/goat and the economic "break-even" price of goat milk decreases (Yazman 1984). Compared to pricing cow milk, it has been determined that the farm gate price for goat milk for a sustainable farm operation must be at least 1.5–2.5 times higher because of higher labor costs. Herd sizes of at least 120 dairy goats with lactation yields of 700 kg/doe maybe needed for an enterprise with an economic future. Labor is a major restraint in dairy management of cows and more so of goats, because of their smaller size and lower volume of milk produced per animal. Total labor requirement in the production of 100 kg goat milk ranges between 57–145 min compared to cows requiring only 7–31 min/100 kg milk production (Kapture 1991). At \$6.00/h labor, 100 kg cow milk requires at most \$3.10 labor cost compared to \$14.50 for the production of goat milk, which is a difference of 4.7 times, explaining the need for the price difference for milk between the two species. Using current U.S. prices for feeds including hay, buck service, veterinary costs, and milking supplies, minus the gain from sale of kids and the fertilizer value of goat manure, but not including fixed overhead, the total production cost of goat milk has been estimated to be about \$70.00/100 kg compared to a current farm gate price for cow milk at \$29.00/100 kg (USDA-ARS 1968; Haenlein 1997). At a yield ratio of 10 kg milk : 1 kg cheese, the milk cost for goat cheese making appears to be at \$7.00/kg cheese. These figures compare with actual prices received on U.S. dairy goat farms for Grade A–3.5% fat goat milk ranging from \$26.00 to \$97.00/100 kg (the later for direct sales at the farm) (Editor 1991), and average commercial prices for goat milk in France at the equivalent of \$51.00/100 kg, in Italy \$47.00, in Spain \$44.00, and in Greece \$36.00 (IDF 2000). Although these countries, and the Mediterranean region in general, are the world's premier countries of successful commercial organization of goat milk, goat cheese, and yogurt production, the average milk production levels per doe are much below those of American dairy goats (Table 69.3), partly because much pasturing is part of their management system. Average annual milk production per doe in France has been at 364 kg, in Spain at 109 kg, in Italy at 104 kg, and in Greece at 73 kg (IDF 2000).

In regions, where most goat meat is supplied by dual purpose dairy or bush goats rather than by single purpose meat goats, a decision must be made by the goat farmer on whether it is more economical to sell most of the available milk and nurse the kids with commercial milk replacer feed, or whether it is more profitable to feed the milk to as many goat kids as possible for sale of kid meat (Haenlein 1997). The decision of alternatively marketing goat milk instead of feeding it for goat meat production can be made on the basis of a formula for R-values (Gall 1990):

$$R = \frac{\text{Kid weight gain (kg)} \times \text{Kid weight price (\$)}}{\text{Milk amount fed (kg)} \times \text{Milk price (\$)}}$$

If R is less than 1.00, then goat milk is more economically sold in the market than fed to kids. If kid weight price is at least more than six times the milk price, then milk feeding for goat meat production is profitable. The formula can also be used to calculate a necessary goat meat price at a given goat milk price, for example, at \$70.00/100 kg milk a break-even price for meat sale would have to be \$4.20/kg for a 25 kg goat kid, that had required 150 kg goat milk to grow to that bodyweight (i.e., 6 kg milk for 1 kg goat meat).

An additional aspect of goat milk pricing is the need for incentive payments to the producer in order to meet and improve health standard requirements for fluid milk, and optimum milk composition for the interests of cheese and yogurt yields. Score cards are used in Europe for the payment of bonus or penalty monies for certain desirable levels of some goat milk contents such as coliform bacteria, listeria, lipolysis, bacteria counts, somatic cell counts (SCC), pH, fat, solids, protein, casein, water, and the absence of antibiotics and sediment. European limits for goat milk to be pasteurized are $<$1.5 million/mL bacteria count, but for raw milk processing $<$0.5 million/mL (IDF 2000). Present SCC limits in goat milk are 1.35 million/mL in France, 1.6 in Spain, 1.743 in Italy, compared to European cow milk SCC limits at 0.4 million/mL. This reflects the research and enforcement dilemma for commercial goat milk production. Officially it has not yet been recognized, that goat milk production is seasonal, which means that average goat milk composition increases in fat, protein and SCC normally without any pathological reasons toward the end of the lactation, which coincides with the autumn season, during which most dairy goats are at the end of their lactation (Haenlein 2002; Borges and others 2004). Any enforcement of the present European cow milk SCC limits to goat milk production is not only not possible, but would be unfair and discriminatory. A separate SCC standard, which takes normal seasonal changes in goat milk composition into account, must be developed. Differential payments for contents of solids are applied by the goat milk powder manufacturer in California, and by commercial processors for goat cheese in several countries as an incentive for higher cheese yields per volume of delivered milk.

The situation of allowing raw goat milk sales for fluid milk consumption and cheese production is quite variable between countries and between states of the United States (Editor 2004), for example, in Arkansas, raw milk sales are illegal, meaning that all milk has to be pasteurized, but on-farm sales directly to the consumer are allowed, as are "incidental sales." The definition for incidental sales is that the monthly number of gallons (3.8 L) of goat milk sold should not be exceeding 100 gallons. A survey of U.S. states found that 29 states allow raw milk sales for human consumption, two states permit raw milk sales with a medical prescription, four states prohibit the sale of raw milk for human consumption, but allow it for animal consumption, and 15

states prohibit the sale of raw milk totally (Editor 2004). Most European countries allow direct farm sales of raw milk, and the organized marketing of certified raw milk according to national regulations, which monitor the presence of pathogenic microorganisms. Such milk is especially popular in France, United Kingdom, and Austria. Thus one can find in the grocery stores of their cities the paper cartons of goat milk with the appropriate labels of certification and nutrient contents for fat, lactose, protein, and energy contents nicely displayed right next to the paper cartons of cow milk, organic milk, and acidophilus milk.

69.2.4 Uniqueness of Goat Milk

The higher price economically necessary for a good goat milk enterprise is also justified by unique quality characteristics of goat milk and its products, which are related to physiological, metabolic, nutritional, and anatomical differences between goats, cows, and sheep. Goats, when pastured, spend more time browsing and less time grazing than cows or sheep, they may walk longer distances, and get along with less frequent water intake. Great differences are also found for goats in the metabolism of minerals, especially molybdenum, copper, iodine, selenium, magnesium, and iron (Haenlein 2001). Unique differences in several important constituents and physical parameters of goat milk, such as proteins, lipids, minerals, vitamins, carnitine, glycerol ethers, orotic acid, enzymes, fat globule size, casein polymorphisms, between goats, cows, and sheep are therefore not surprising, and are significant for goat milk in human nutrition, justifying its higher price.

69.3 CHEMICAL COMPOSITION OF GOAT MILK

69.3.1 General Compositional Characteristics of Goat Milk

Proximate composition of goat milk generally resembles that of cow milk, although the former contains more fat, protein and minerals, and less lactose and casein than the latter (Table 69.5). Several reports (Parkash and Jenness 1968; Jenness 1980; Haenlein and Caccese 1984; Juarez and Ramos 1986; Park 1991) have shown general and detailed chemical composition of goat milk. As known in cow milk, composition of goat milk is

TABLE 69.5 Comparison of Average Composition of Basic Nutrients Among Goat, Cow, and Human Milk.

Composition	Goat	Cow	Human
Fat, %	3.8	3.6	4.0
Solid-not-fat, %	8.9	9.0	8.9
Lactose, %	4.1	4.7	6.9
Protein, %	3.4	3.2	1.2
Casein, %	2.4	2.6	0.4
Albumin, globulin, %	0.6	0.6	0.7
Non-protein N, %	0.4	0.2	0.1
Ash, %	0.8	0.7	0.3
Calories/100 mL	70	69	68

Source: Posati and Orr (1976), Jenness (1980), and Haenlein and Caccese (1984).

also expected to be varied with diet, breed, animals within breed, parity, environmental conditions, feeding and management conditions, season, locality, and stage of lactation (Schmidt 1971; Underwood 1977). Some of the variations in composition may be related to the lack of standardized dairy production practices in the dairy goat industry and difficulty in procuring milk samples representing pooled bulk milk from various herds and farms (Chandan and others 1992).

On average, cow milk contains about 12.2% total solids (3.2% protein, 3.6% fat, 4.7% lactose, and 0.7% ash), while goat milk contains about 12.1% total solids (3.4% protein, 3.8% fat, 4.1% lactose, and 0.8% ash) (Table 69.5; Posati and Orr 1976; Haenlein and Caccese 1984). These compositional figures are only average values which do not represent individual animals, and considerable differences exist between breeds and among individual goats.

The lower casein in goat milk compared to cow milk is closely related to coagulation property and yield of curds during cheese manufacture. Goat and cow milks contain substantially higher levels of protein, casein, calcium, phosphate and ash than human milk (Table 69.5). This difference in compositional characteristics of milk is believed to be strongly correlated with the growth rates of the young between different species, where the newborn of goats and cows grow a lot faster than human infants.

69.3.2 Factors Affecting Composition and Physicochemical Properties of Goat Milk

69.3.2.1 Diet. The effect of diet on milk composition appears to be similar in both goat and cow milk, although some variations of results have been observed (Juàrez and Ramos 1986). An increase in the energy content of the diet in high-yield goat breeds during lactation tended to augment milk production and reduced the fat content (0.2–0.4%) while raising the nitrogen content (0.1–0.15%) (Fehr and Le Jaouen 1976; Juàrez and Ramos 1986). Merlin and others (1988) reported that type of feed affected the total protein content of milk, whereas fat content was not changed. Water deprivation for 48 h resulted in reduction of milk yield, and higher lactose and protein contents (Dahlborn 1987).

The restricted roughage and high concentrate diets are accompanied by a decrease in the level of dietary fiber. The entire diet of the cow has to contain at least 17% crude fiber to prevent a depression in milk fat (Schmidt 1971). The role of dietary crude fiber levels on milk fat depression was also observed in goat milk by Calderon and others (1984). In a review on the effects of nutritional manipulation on composition and yield of goat milk, Morand-Fehr and Sauvant (1980) reported that goats fed a high concentrate diet (about 15% of their total requirements from concentrates) underwent nearly a 20% increase in milk yield, with slight decrease in fat content and increases in lactose and protein contents. Depression of fat content was similar to that for dairy cows, and there were no differences in either milk protein or total solids composition with the high-concentrate diet. Low-fat diets resulted in a drop in the milk fat content (Le Jaouen 1972), while adding protected lipids led to an appreciable increase in milk fat content (Morand-Fehr and Flamant 1983). However, high protein diets did not change the nitrogen content of the goat or cow milks (Vignon 1976). Increasing the protein content of the diet above the normal recommended standards had no effect on yield but caused only a slight increase in the nonprotein nitrogen content of cow milk (Juàrez and Ramos 1986).

69.3.2.2 Breed. The Saanen is best known as the Holstein of the goat world, producing a high quantity of milk with somewhat low fat levels (Haenlein and Caccese 1984). The other extreme case is the Nubian breed, which is equivalent to the Jersey breed of cows. Nubians produce less milk with higher solids, including fat and solids-not-fat (SNF). The Toggenburg, LaMancha, Oberhasli, and Alpine breeds produce milk yield and composition in between the Saanen and Nubian (Haenlein and Caccese 1984).

Variation in the fat content among the different breeds is greater than variation in protein content (Ramos and Juàrez 1981). The fat and total solids contents of the milk of imported Alpine, Saanen, and Anglo-Nubian breeds in tropical environments were lower than those of the same breeds raised under temperate climate conditions, which might be attributed to both improper diet and the higher temperatures (Juàrez and Ramos 1986). Indigenous breeds (e.g., West African Dwarf and Red Sokoto) have much richer composition but lower yields compared to those of the Swiss breeds.

69.3.2.3 Stage of Lactation. The milk production of the dairy goat at kidding starts out at a relatively high level and continues to increase to a peak approximately 3–4 weeks after freshening (Fig. 69.1; Le Jaouen 1987), where a similar trend with a peak at 3–6 weeks would occur in dairy cows (Schmidt 1971). The high production may be held for a few weeks, then the milk yield gradually declines toward the end of lactation (Fig. 69.1).

The fat, solids-not-fat, and protein contents of cow milk are high in early lactation, fall rapidly and reach a minimum during the second to third months of lactation, and then increase towards the end of lactation (Schmidt 1971). This results in an inverse relationship between the yield of milk and percentage composition of these components (Fig. 69.1). In goat milk, fat and protein contents decrease from onset of lactation to the fourth or fifth month and then remain low for a variable length of time, increasing at the end of lactation. Lactose content in both goat and cow milk shows some fluctuations during lactation, which is different from fat and protein contents, being first low, rising in mid lactation and decreasing again at the end of lactation (Renner 1982; Larson 1985).

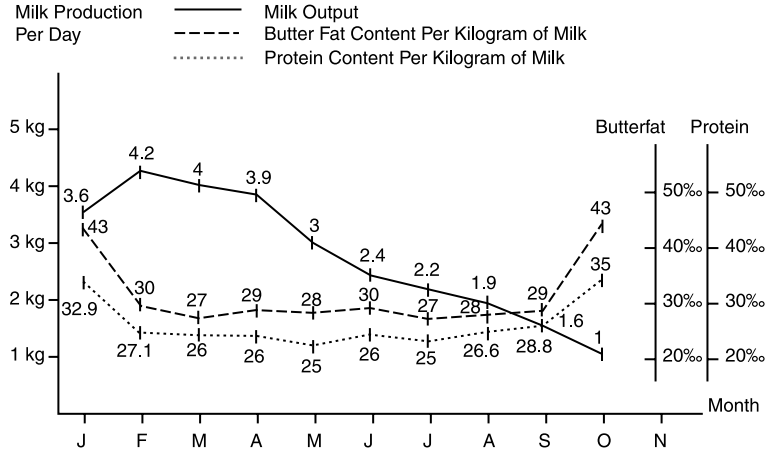

Figure 69.1 *Changes in milk yield, fat and protein contents during different stages of lactation in goat milk (Le Jaouen 1987).*

69.3.2.4 Season. Fat and SNF levels in goat milk vary with season (Fig. 69.1). In the temperate climate, late summer milk contains lowest fat and SNF contents (Parkash and Jenness 1968; Chandan and others 1992). Fat content of milk may vary as much as 2% and SNF as much as 1%, depending on the season. A Canadian study demonstrated that cheese yield varied directly with seasonal deviations in levels of fat and protein in milk (Irvine 1974).

Natural breeding usually results in goats freshening at the beginning of spring, although artificial breeding methods are practiced for year-round milking. The seasonal variations in milk composition are concommitantly involved with lactation stages in overall observed fluctuations of milk constituents (Chandan and others 1992).

69.4 CHEMICAL CHARACTERISTICS OF GOAT MILK COMPARED TO OTHER SPECIES MILK

69.4.1 Lipids

69.4.1.1 General Composition of Goat Milk Lipids. Goat milk is considered a naturally homogenized milk, where its fat globule size is smaller than in cow milk. Average diameters of fat globules for goat, cow, buffalo and sheep milks were reported to be 3.49, 4.55, 5.92, and 3.30 μm, respectively (Fahmi and others 1956). Smaller fat globules of goat milk make a better dispersion and a greater surface area of the fat for better digestion by lipases. The percent fat of goat milk is comparable to cow milk, but the physical and chemical structures are significantly different between the two species.

For gross characterization of milk fat, goat milk contains 97–99% of free lipids and 1–3% bound lipids of total milk fat (Table 69.6). The ratio of bound to free lipids is comparable to that for cow milk (Cerbulis 1982). Goat milk has similar fractional compositions of free lipids compared to cow milk. Free lipids of goat milk contained 96.8% triglycerides, 2.2% diglycerides, and 0.9% monoglycerides, whereas bound lipids contained 46.8% neutral lipids and 53.2% polar lipids (8.5% glycolipids and 44.7% phospholipids). Quantitative analysis of the phospholipid fraction of bound lipids of goat milk revealed that it had 35.4% phosphatidyl ethanolamine, 3.2% phosphatidyl serine, 4.0% phosphatidyl inositol, 28.2% phosphatidyl choline, and 29.2% sphingomyelin (Cerbulis and others 1982).

Most cholesterol in goat milk, as in cow milk, is in a free state (52 mg/100 g fat) with a small portion in ester forms, which constitutes less than 4% of the total cholesterol (Jenness

TABLE 69.6 Quantitative Distribution of Lipids in Bound and Free Fractions of Goat Milk.

Lipid Components	% Total Lipid
Free lipids	97–99%
Triglycerides	96.8
Diglycerides	2.2
Monoglycerides	0.9
Bound lipids	1–3%
Neutral lipids	46.8
Glycolipid	8.5
Phospholipid	44.7

Source: Cerbulis and others (1982).

TABLE 69.7 Fatty Acid Composition of Goat Milk Lipids.

	Lipids in Fat Globule Membrane					
Fatty Acid	Triglyceride (wt%)	Free Fatty Acid (wt%)	Total Lipid (wt%)	Cholesterol Esters (wt%)	Phosphatidyl Ethanolamine (mole%)	Phosphatidyl Serine (mole%)
C4 : 0			4.5			
C6 : 0			3.2			
C8 : 0			3.4			
C10 : 0	0.45	0.6	9.8	5.2		
C10 : 1	–	–	4.1	Tr		
C12 : 0	1.00	1.5	–	4.2		Tr
C12 : 1	–	–		1.0		
C14 : 0	4.32	3.93	8.7	9.2	0.6	4.1
C14 : 1	1.34	0.87		1.4		
C15 : 0	1.54	0.59		1.3		
C16 : 0	26.3	30.6	21.9	39.3	10.0	38.7
C16 : 1	3.29	3.23	1.8	Tr		
C17 : 0	2.49	2.34	0.9	Tr		
C18 : 0	31.6	30.4	8.3	9.0	9.9	8.7
C18 : 1	20.7	23.0	20.8	26.5	52.0	32.2
C18 : 2	2.55	1.34	3.2	2.1	18.4	7.1
C18 : 3	1.72	1.69			1.7	
C20 : 0	1.91	–			2.8	1.2
C20 : 1	–	–			4.5	1.0
C21 : 0	–	–				3.1
C22 : 0	0.85	–				

Source: Jenness (1980), Chandan and others (1992).

1980; Chandan and others 1992). Fatty acid composition of cholesterol esters reveals that goat cholesterol esters have greater palmitic and oleic acid fractions than the cow counterparts (Table 69.7) (Jenness 1980; Juàrez and Ramos 1986). Average 66% free and 42% of esterified cholesterol was associated with goat milk fat globules (Keenan and Patton 1970). The level of unsaponifiable matter in goat milk is 24 mg/100 mL or 46 mg/100 g fat, which is comparable to that in cow milk (Arora and others 1976).

69.4.1.2 Fatty Acid Composition of Caprine Milk. Caprine milk has significantly higher levels of short and medium chain length fatty acids (MCT) (C4 : 0–C14 : 0) than cow and human milks (Table 69.7; Jenness and Patton 1976; Jenness 1980; Juàrez and Ramos 1986; Jensen and others 1990; Haenlein 1992). Comparative study on fatty acids composition of total lipids showed that goat milk contains almost twice as much capric, caproic, caprylic, and lauric acids than cow milk, which is correlated to "goaty" flavor in goat milk (Jenness 1980; Haenlein and Caccese 1984; Juàrez and Ramos 1986). (Table 69.7). Goat milk has a unique characteristic in the lauric : capric fatty acid (C12 : C10) ratio, where caprine milk is significantly lower in this ratio than cow milk (0.46 vs. 1.16) (Iverson and Sheppard 1989).

In light of long chain fatty acids, both goat and cow milk fats contain remarkably high concentrations of C16 : 0 and C18 : 1 (oleic), which are common to most mammals (Table 69.7). Levels of long-chain fatty acids (C16 : 0, C18 : 0, and C18 : 2) in goat milk were significantly different between milking herds (Alonso and others 1999). Five branched-chain fatty acids (BCFA) (*iso*- and *anteiso*-C15 : 0, *iso*- and *anteiso*-C17 : 0, and *iso*-C16 : 0) with $>0.1\%$ of the total fatty acid methyl esters and another 31 (the most monomethylated) with $<0.1\%$, including 4-ethyloctanoate were identified in caprine milk. Numerous BCFA (all having more than 11 carbons) were identified and quantified (Massart-Leen and others 1981), and over 20 volatile BCFA were identified in caprine cheese (Ha and Lindsay 1991). Caprine milk fat has a range of other monomethyl-branched components, mostly with methyl-substitution on carbons 4 and 6, which are virtually absent from cow milk with only a trace amount of 6-methyl-hexadecanoate detected (Alonso and others 1999).

The positional isomers of cis- and trans-octanoate in goat milk fat were 86% of the cis-C18 : 1 in oleate ($\triangle 9$) form, as opposed to 96% in cow milk (Jensen 1973; Jenness 1980). Both goat and cow milk fat contain adequate amounts of essential fatty acids for human infants.

Conjugated linoleic acid (CLA) has gained a great attention in recent years because of its several beneficial effects on health. The CLA content of goat milk can be increased by dietary manipulation and supplementation with certain ingredients such as addition of seed oil. Feeding canola oil at 2% and 4% of grain intake to Alpine does, compared to the non-treated control group, increased CLA in milk by 88% and 210%, respectively (Mir and others 1999).

69.4.1.3 Free Fatty Acids in Caprine Milk. Free fatty acid (FFA) is measured by acid degree value (ADV), which is a measure of lipolysis or degree of formation of FFA in milk and dairy products. Goat milk has higher sensitivity to spontaneous lipolysis than cow milk due to the difference in lipase distribution. Free fatty acid content of goat milk is 3.11 μeq/mL compared with cow milk (3.0 μeq/mL) and buffalo milk (3.4 μeq/mL) (Agnihotri and Prasad 1993).

Goat, sheep, and cow cheeses had similar FFA profiles, with the exception of 4-ethyloctanoic acid which was present in goat and sheep cheese, but was absent in

cow cheese (Ha and Lindsay 1991). The 4-methyloctanoic acid content in goat Cheddar cheese increased significantly from day 1 to week 12 of the aging period (Attaie and Richter 1996). The threshold concentration of 4-ethyloctanoic acid for goaty aroma was 1.8 ppb (Boelens and others 1983) and 6.0 ppb (Brennand and others 1989) in diluted citric acid solution at pH 2.0.

FFA fraction of goat milk has been shown to be related to "goaty" flavor intensity in the milk. A positive correlation was found between goaty flavor and free fatty acids (5.6 and 2.7 meq/L in samples stored for strong and weak flavor) (Bakke and others 1977). Concentration of FFA increased during storage at 4°C, where the FFAs initially consisted of short chain acids, then long chain FFA such as C16 : 0 and C18 : 0 increased after 10 days of storage (Bas and others 1978).

69.4.2 Proteins

69.4.2.1 Major Proteins and Caseins in Goat Milk. Goat milk has six principle proteins: β-lactoglobulin (β-Lg), α-lactalbumin (α-La), κ-casein (κ-CN), β-casein (β-CN), α_{s1}-casein (α_{s1}-CN), and α_{s2}-casein (α_{s2}-CN) (Jenness 1980; Haenlein and Caccese 1984; Carles 1986; Mikkelsen and others 1987; Park 2006). These goat milk proteins were named after their corresponding cow milk proteins because of their homologous nature of both species milks in composition and properties (Whitney and others 1976). The casein composition in goat milk is influenced by genetic polymorphism on the casein loci as described in the following section (Tziboula-Clarke 2003).

β-casein is shown to be the major component of the casein fraction in goat milk, whereas α_{s1}-casein is the major casein in cow milk. Total casein content of goat milk is slightly lower than that of cow milk (Table 69.8). Goat milk has markedly different levels of α_{s1}- and α_{s2}-CN from those levels of cow milk, where goat milk has much lower α_{s1} and higher α_{s2}-CN than cow milk (Chandan and others 1968; Remeuf and

TABLE 69.8 Comparison of Concentrations of Protein Moieties Among Goat, Cow, and Human Milks.

Proteins	Goat	Cow	Human
Protein (%)	3.5	3.3	1.2
Total casein (g/100 mL)	2.11	2.70	0.40
α_{s1} (% of total casein)	5.6	38.0	–
α_{s2} (% of total casein)	19.2	12.0	–
β (% of total casein)	54.8	36.0	60–70.0
κ (% of total casein)	20.4	14.0	7.0
Whey protein (%) (albumin and globulin)	0.6	0.6	0.7
Nonprotein N (%)	0.4	0.2	0.5
Immunoglobulin			
IgA (milk: μg/mL)	30–80	140	1000
IgA (colostrum: mg/mL)	0.9–2.4	3.9	17.35
IgM (milk: μg/mL)	10–40	50	100
IgM (colostrum: mg/mL)	1.6–5.2	4.2	1.59
IgG (milk: μg/mL)	100–400	590	40
IgG (colostrum: mg/mL)	50–60	47.6	0.43

Source: Jenness (1980), Remeuf and Lenoir (1986), Renner and others (1989), Chandan and others (1992), and Park and Haenlein (2004).

Lenoir 1986). However, goat milk shows considerable variations in its α_{s1}-CN content ranging from 2.7 g/L to only 0.12 g/L (Mora-Gutierrez and others 1991), which is genetically regulated in different breeds.

69.4.2.2 Whey Proteins. While caseins are the major proteins in milk, whey or serum protein fractions consist of mainly three components, which are bovine serum albumin, β-Lg, and α-La. Whey proteins also include some immunoglobulins and proteose-peptone fractions (Whitney and others 1976; Jenness 1980). β-Lg and α-La are the two whey proteins generally contained in significant quantities.

It was reported that β-Lg contents are similar in the two species milks, but goat milk contains nearly twice as much α-La as cow milk (Jenness 1980). However, Storry and others (1983) indicate that the α-La content in the two species is approximately equal but the β-Lg content in goat milk is practically double the α-La content.

β-Lg in goat milk has been separated and sequenced (Juàrez and Ramos 1986). Goat β-Lg has three less-negatively charged and one more positively charged residues than bovine β-Lg at pH values of 5–9 (Jenness 1982). This difference in ionizable groups may expound the difference in titration curves previously noted for the two proteins and the slower electrophoretic mobility of goat β-Lg at alkaline pH levels (Juàrez and Ramos 1986).

Goat α-La has been confirmed to be similar to that of the bovine homolog by various optical analyses, where the two species proteins have equal exposure of Tyr, Trp, and Lys groups in their conformation (Jenness 1980). α-La is present in all milks which contain lactose, since it is required for biosynthesis of lactose at meaningful rates (Ebner and Schanbacher 1974). α-La is considered best as a modifier protein, since it changes the apparent K_m of the substrate, glucose, and does not appear to participate directly in the catalytic reaction (Ebner and Schanbacher 1974). Four disulfide bonds are found in α-La as the major component, and the isolation of an α-La with three disulfide bonds from an α-La B preparation accounts for approximately 5% of the total α-La (Brown and others 1969).

69.4.2.3 Amino Acid Composition. The characteristics of the amino acid compositions in goat milk proteins show that differences between casein fractions are much greater than differences between species (goat vs. cow) within a casein fraction (Park 2006). The α-CN contains more aspartate, lysine, and tyrosine than β-CN, while the latter has more leucine, proline, and valine contents than the former. The β-Lg contains significantly less aspartate than α-La, whereas the opposite trend is noted for alanine and glutamate concentrations (Davis and others 1994).

Total amino acid contents in goat and other nonprimate milks were substantially greater than those in human and primate milks (Davis and others 1994). Other commonalities in all species milks were essential amino acids (EAA) 40%, branch-chain amino acids (BCAA) 20%, and sulfur amino acids 4% of the total amino acids. The EAA contents of goat and cow milk were greater than those of human milk, whereas the opposite trend was observed for the BCAA contents (Davis and others 1994). Goat, cow, and human milks have a satisfactory balance of EAA equalling or exceeding the FAO-WHO requirements for each amino acid for human infants (Jenness 1980).

69.4.2.4 Nonprotein Nitrogen and Other Nitrogen Moieties of Goat Milk. Nonprotein nitrogen (NPN) contents in goat and human milk are much higher than in cow milk (Park 1991; Table 69.9). In comparison with cow milk, goat milk has

TABLE 69.9 Concentration of Total N, NPN, and Phosphate in Natural Goat and Cow Milk and Soy-Based Infant Formulae[1].

		Total N		NPN		P_2O_5	
Milk Group	N[2]	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Goat milk							
Alpine	25	0.390[c]	0.032	0.048[b]	0.008	0.166[a]	0.020
Nubian	25	0.556[a]	0.013	0.061[a]	0.013	0.212[a]	0.015
Cow milk							
Holstein	25	0.392[c]	0.058	0.033[c]	0.002	0.173[a]	0.022
Jersey	25	0.505[b]	0.043	0.038[c]	0.004	0.211[a]	0.118
Formula milk							
Brand A	5	0.227[d]	0.026	0.020[d]	0.003	0.211[a]	0.008
Brand B	5	0.259[d]	0.016	0.019[d]	0.003	0.192[a]	0.053

[a,b,c,d]Means with different superscripts within same column are different ($P < 0.01$).
[1]Expressed in g/100 mL.
[2]Number of determinations per mean value.
Source: Adapted from Park (1991).

a higher NPN, 8.7% as opposed to 5.2%, and a lower proportion of coagulable proteins and caseins, 70.9% and 75.6% compared to 73.0% and 77.8%, respectively (Grappin and others 1981). Similar values of casein content (% of total nitrogen) were reported by Jenness (1980). True protein is calculated as crude protein minus NPN, where the ratios of casein to true protein for goat and cow milks are 82.7% and 82%, respectively.

The composition of NPN indicates, that it contains several nitrogenous compounds, and its components (mg N/100 mL) in cow milk include: 0.17 ammonia N, 6.54 urea N, 0.19 creatinine, 3.55 creatin, 1.55 uric acid, 2.20 α-amino N, and 5.63 unaccountable N, respectively (Rowland 1937; Jenness and Patton 1976). NPN contents in goat and cow milk also are different between different breeds, indicating that Nubian has higher NPN levels than Alpine goats, like the Jersey cow has higher NPN than the Holstein cow milk (Table 69.9; Park 1991, 1992a).

69.4.3 Minor Proteins, Enzymes, and Other Constituents

Goat milk has comparable levels of lactoferrin, transferrin, and prolactin to those of cow milk (Table 69.10). Human milk contains more than 2 mg lactoferrin/mL, which amounts to being 10–100-fold higher than in goat milk. Goat and cow milk contain transferrin levels of 20–200 μg/mL, while human milk contains <50 μg/mL. Goat milk has higher levels of folate-binding protein than cow milk, causing actual folate content being lower in the former than the latter (Table 69.10; Ford and others 1972; Renner and others 1989; Chandan and others 1992). The protease activity in fresh goat milk is greater than in cow milk (Anjaneyulu and others 1985).

Goat milk has different distributions of enzymes compared to cow milk (Table 69.10; Chilliard and others 1984; Chandan and others 1992). Lysozyme concentrations of goat and cow milks are comparable (Table 69.10), where goat milk contains in average 25 μg of lysozyme, 425 μg of ribonuclease, and 36 μM of lipase/100 mL (Chandan and others 1968). Ribonuclease level in cow milk is much greater than in

TABLE 69.10 Minor Proteins, Enzymes, and Other Constituents in Goat Milk.

Constituents	Concentration Ranges
Lactoferrin	20–200 μg/mL
Transferrin	20–200 μg/mL
Prolactin	44 ng/mL
Folate-binding protein	12 μg/mL
Lysozyme	25 μg/100 mL
Ribonuclease	425 μg/100 mL
Lipase	36 μM/100 mL
Lactate dehydrogenase	47 μM/s/mL
Malic dehydrogenase	50 μM/s/mL
Xanthine oxidase	19–113 μL O_2/h/mL
Alkaline phosphatase	11–13 mg/L
Orotic acid	13 mg/L
Carnitine	16.4 mg/L
ATP	19 mg/L
Free amino acid	48 mg/L
Sialic acid protease	13.89 mg/L

Source: Chandan and others (1968), Jenness (1980), Renner and others (1989).

goat milk, where this enzyme is identical to bovine pancreatic ribonuclease (Juàrez and Ramos 1986).

Alkaline phosphotase level of goat milk ranged from 11 to 13 mg/L. Some researchers reported that this enzyme can be inactivated at around 45°C. These reports indicate that the effectiveness of the alkaline phosphatase test for pasteurization of goat milk may not be as good as generally assumed from cow milk (Juàrez and Ramos 1986).

Xanthine oxidase activity of goat milk is less than 10% of that of cow milk (Chandan and others 1992). Xanthine oxidase has been implicated in the spontaneous development of undesirable oxidized flavors in market milk and other dairy products, and interest in this enzyme has increased, because of its possible involvement in development of atherosclerosis in humans (Juàrez and Ramos 1986). Caprine xanthine oxidase contains higher amounts of aspartic acid, glutamic acid, proline, and glycine and lower amounts of serine than bovine xanthine oxidase (Zikakis and others 1983).

Lipase activity in goat milk is significantly correlated with spontaneous lipolysis, which may be attributed to its specific lipolytic system. Significantly lower lipoprotein lipase activity was observed in fresh goat milk cooled to 4°C, compared to the enzyme activity in cow counterparts. Lipases play a major role in flavor development in milk and dairy products during milk processing and storage.

Goat milk has in average 47 μ moles/s/mL of lactic dehydrogenase and 50 μ moles/s/mL of malic dehydrogenase (Jenness 1980).

69.4.4 Carbohydrates

The major carbohydrate of goat milk is lactose. Average lactose content of goat milk is 4.45%, which is about 0.2–0.5% less than that of cow milk which is 4.66% (Posati and Orr 1976; Chandan and others 1992). Cow milk contains insignificant levels of monosaccharides and oligosaccharides, while their presence in goat milk is not known (Chandan and others 1992).

69.4.5 Minerals

Goat milk contains higher calcium, phosphorus, potassium, magnesium, and chlorine, and lower sodium and sulfur contents than cow milk (Table 69.11; Chandan and others 1968; Haenlein and Caccese 1984; Park and Chukwu 1988, 1989). Human milk contains only one-quarter to one-sixth of calcium and phosphorus. Although the fluctuation of macromineral contents may not be considerable in goat milk, their levels can vary, depending on the breed, diet, animal and stages of lactation (Park and Chukwu 1988; Park 2006).

A close inverse relationship was observed between lactose content and the molar sum of sodium and potassium contents of goat or other species milks (Konar and others 1971; Park and Chukwu 1988). Chloride is positively correlated with potassium and negatively with lactose, but sodium is not significantly correlated with K, Cl, and lactose (Park 2006).

Unlike most of macrominerals, levels of trace minerals are affected by diet, breed, animals, and stages of lactation (Park and Chukwu 1989). Mean concentrations of Mn, Cu, and Fe in French-Alpine goat milk were 0.33, 5.0, and 1.7 mg/L, while Anglo-Nubian goat milk contained significantly higher levels of Cu (1.36 vs. 1.69 mg/L) and Zn (7.9 vs. 11.9 mg/L) (Park and Chukwu 1989). In Norwegian bulk goat milk, a positive correlation was observed between levels of Co and P, K, Na, Ca, Al, and Mg (Brendehaug and

TABLE 69.11 Comparison of Mineral and Vitamin Contents Among Goat, Cow, and Human Milks.

Composition	Goat	Cow	Human
Minerals			
Calcium (CaO), %	0.19	0.18	0.04
Phosphorus (P_2O_5), %	0.27	0.23	0.06
P_2O_5/CaO	1.4	1.3	1.4
Chloride (g/100 mL)	0.15	0.10	0.06
Fe (μg/mL)	0.7	0.8	2.0
Mg (mg/100 mL)	16	12	4
K (mg/100 mL)	182	152	55
Na (mg/100 mL)	41	58	15
Cu (mg/100 mL)	0.05	0.06	0.06
Mn (mg/100 mL)	0.032	0.02	0.07
Zn (mg/100 mL)	0.56	0.53	0.38
Se (μg/100 mL)	1.33	0.96	1.52
Mo (μg/100 mL)	1.24	2.59	–
Vitamins			
Vitamin A (IU/g fat)	9	21	32
Thiamin (mg/100 mL)	0.05	0.04	0.014
Riboflavin (mg/100 mL)	0.14	0.16	0.04
Pantothenate (mg/100 mL)	0.31	0.32	0.20
Vitamin B_6 (mg/100 mL)	0.05	0.04	0.01
Folic acid (μg/L)	6	50	50
Vitamin B_{12} (mg/100 mL)	0.065	0.357	0.03
Vitamin C (mg/100 mL)	1.29	0.94	5.00
Vitamin D (IU/g fat)	0.7	0.7	0.3

Source: Posati and Orr (1976), Haenlein and Caccese (1984), Park and Chukwu (1989), Chandan and others (1992), and Park and Haenlein (2006).

Abrahamsen 1987). Zinc content is the highest among trace minerals, and Zn contents in goat and cow milks are greater than in human milk (Park and Chukwu 1989). Iron contents of goat and cow milks are significantly lower than in human milk (Table 69.11).

The respective average Mo contents of goat and cow milks are 12.4 and 25.9 μg/L (Table 69.11) (Hart and others 1967). The supplementation of 1.1 mg Mo/day in goat's diet produced 12 μg/L of Mo in milk, while 13.0 mg Mo/day elevated Mo in milk to approximately 70 μg/L (Jenness 1980). Selenium is an essential trace mineral. It is a component of glutathione peroxidase, which detoxifies peroxide. Selenium deficiency is correlated with liver necrosis, abnormal cardiac function, and even cancer (Debski and others 1987; Park 2006). Goat and human milk contain higher concentrations of selenium than cow milk (Table 69.11). Less than 3% of the total selenium is associated with the lipid fraction of milk. Glutathione peroxidase was higher in goat milk than in human and cow milk. (Debski and others 1987).

69.4.6 Vitamins

Goat milk supplies adequate amounts of vitamin A and niacin, and excesses of thiamin, riboflavin, and pantothenate for a human infant (Table 69.11; Parkash and Jenness 1968; Ford and others 1972). Goat milk has higher amounts of vitamin A than cow milk. Since goats convert all β-carotene into vitamin A in the milk, caprine milk is

whiter than most bovine milk. Vitamin B in goat and cow milks is synthesized in the rumen, whereby the B levels may be independent of diet (Mann 1988).

Compared to cow milk, goat milk is deficient in folic acid and vitamin B_{12} (Collins 1962; Davidson and Townley 1977; Jenness 1980; Haenlein and Caccese 1984; Park and others 1986). Cow milk has five times more folate and vitamin B_{12} than goat milk, where folate is necessary for the synthesis of hemoglobin (Collins 1962; Davidson and Townley 1977). However, both goat and cow milks are deficient in pyridoxine (B_6), vitamin C and vitamin D, where these vitamins must be supplemented from other food sources (McClenathan and Walker 1982).

Lavigne and others (1989) showed that high temperature and short-time (HTST) pasteurization method was the best processing method for goat milk to preserve various vitamins as well as extend the shelf-life. The HTST, flash, and UHT process were better than LTLT and autoclave treatment methods in preservation of thiamine, riboflavin, and vitamin C in goat milk (Lavigne and others 1989).

69.5 PROTEIN POLYMORPHISM AND RENNETABILITY OF GOAT MILK

69.5.1 Protein Polymorphism in Goat Milk

It has been shown that a direct relationship exists between protein allelic variants and differences in casein (CN) content, and in physicochemical properties of milk (Jordana and others 1996). Milk protein polymorphisms are caused either by the substitution of amino acids or by the deletion of several of them, and they can be detected through electrophoresis of milk and/or analysis of DNA (Moioli and others 1998).

An association has been established between the existence in the Saanen and Alpine dairy breeds of at least seven alleles and various amounts of α_{s1}-CN in the milk (Tables 69.8 and 69.12) (Grosclaude and others 1987; Mahe and Grosclaude 1989). Polymorphism of α_{s1}-casein controls the level of α_{s1}-casein excretion in milk, and more than 18 allelic genotypes have been identified in goat milk (Tziboula-Clarke 2003). Each one of the alleles α_{s1}-CN^A, α_{s1}-CN^B, and α_{s1}-CN^C contribute approximately 3.6 g CN/L milk, whereas α_{s1}-CN^E contributes only 1.6 g, α_{s1}-CN^F, and α_{s1}-CN^G 0.5 g, and α_{s1}-CN^0 appears to be a null allele (Jordana and others 1996). The allele E (medium) is related to an intermediate content, and those F and G (weak) are associated with low contents of α_{s1}-casein (0.5 g/L per allele) (Tziboula-Clarke 2003).

Grosclaude and others (1987) reported that the relationship between total casein content (TC) and α_{s1}-CN content can be calculated as $TC = 19.59 + 0.64\ \alpha_{s1}$. Quantitative variations of α_{s1}-CNs were also reported in Italian goat breeds in relation to the coagulation properties of the goat milk (Ciafarone and Addeo 1984; Ambrosoli and others 1988). In characterization of the six caprine α_{s1}-CN variants, Brignon and others (1990) found that variants A and B had the closest homology with the ovine and bovine sequences. The higher amount of α_{s1}-CN is likely to be associated with alleles α_{s1}-CN^A, α_{s1}-CN^B, and α_{s1}-CN^C in goats. These alleles are normally high in caprine species, while the other alleles are defective mutants. The decreased rate of α_{s1}-CN synthesis associated with allele α_{s1}-CN^F is due to altered RNA splicing, as a consequence of an exonic point deletion (Leroux and others 1992).

Considering the economic contribution of dairy goat production in France, Spain, Italy, Greece, and other countries, it would be important to evaluate the gene frequency of

TABLE 69.12 Protein Variants in Goats.

Locus	Protein Variants	DNA Polymorphisms
α_{s1}-Casein	A, B_1, B_2, B_3,C, D, E, F, G, O	A, B, C, D, E, F, G, O
α_{s2}-Casein	A, B, C	*Eco*RI and *Eco*RV polymorphic patterns by using a cDNA bovine probe
β-Casein	A, B, O	Point mutation in exon 7
κ-Casein	A, B	*Bam*HI, *Eco*RV, and *Pvu*II polymorphic patterns by using a cDNA bovine probe
α-Lactalbumin	A, B	Nothing existing
β-Lactoglobulin	A, B	Nothing existing

Source: Moioli and others (1998).

α_{s1}-CN alleles in different dairy goat breeds, as well as to determine the effect of the α_{s1}-CN genotype as criterion in selection programs.

69.5.2 Variants and Alleles at the Milk Protein Loci

There are six major proteins found in goat and sheep milk, and caseins are nearly 80% of the total protein amount. Four types of caseins α_{s1}, α_{s2}, β, and κ are mainly affecting cheese yield and quality (Moioli and others 1998). The genes coding for the four caseins are closely linked, where the heritable transmitted unit is the haplotype (Grosclaude 1988). The major whey proteins in goat and cow milk are α-lactalbumin and β-lactoglobulin.

In most European dairy goat breeds such as Alpine, Saanen, Poitevine, Garganica, Maltese, Murciana-Granadina, Malaguena, a polymorphism with ten alleles (A, B_1, B_2, B_3, C, D, E, F, G, O) was found (Table 69.12) (Martin 1993; Jordana and others 1996; Moioli and others 1998; Tziboula-Clarke 2003). The α_{s1}-CNs A, B, C, and E differ only due to a few amino acid substitutions, while α_{s1}-CNs D and F show considerable structural differences, consisting in internal deletions of 11 and 37 residues respectively, which include the multiple phosphorylation sites (Martin 1993).

69.5.3 Genetic Typing

Milk protein content ranges between 2.5% and 4.4% in most dairy goats. The four caseins (CN) such as α_{s1}-CN, α_{s2}-CN, β-CN, and κ-CN, are the main components accounting in ruminants for 76–86% of the total protein (Ciafarone and Addeo 1984; Swaisgood 1992).

At DNA level, polymorphisms are due either to point mutations or to DNA rearrangement phenomena (Moioli and others 1998). For point mutations, such as single nucleotide substitution, differences between two individuals are detected using restriction enzymes which cut DNA only at a specific DNA sequence: different alleles will be displayed by the electrophoresis gel with bands of different lengths (Moioli and others 1998). When the point mutation is not recognized by any endonucleases, allele-specific primers can be built for the two different variants and used independently in a polymerase chain reaction (PCR) with a second common primer to amplify the region where the mutation has occurred (David and Deutch 1992).

For genetic typing, rearrangement phenomena consist of insertions, deletions and inversions of DNA fragments (Moioli and others 1998). The most common are microsatellites, which consist of repeated sequences of two, three, or four nucleotides (Moioli and others 1998). Specific microsatellites contained within a stretch of sequenced unique DNA can be individually amplified by means of the PCR (polymerase chain reaction) using a pair of flanking unique oligonucleotides (Soller 1990). Amplified sequences produced from different individuals exhibit highly polymorphic length variation and are used to construct linkage maps by estimating recombination frequencies from individuals belonging to families.

69.5.4 Effect of Milk Protein Polymorphism on Renneting Properties

Goat milk has a poorer coagulating ability, which is due essentially to its lower casein content and to specific properties of casein micelles as composition, size, and hydration. On average, the firmness of the gel of goat milk is clearly lower, and the gel from goat milk with an equal casein content is not as firm as from cow milk (Storry and others 1983). Proportions of the four caseins (α_{s1}, α_{s2}, β, and κ) are not the same in the two milks and there are great variations of α_{s1}-casein content in individual goat milks due to the occurrence of genetic polymorphism of this casein, which may have great repercussions on their cheesemaking properties (Remeuf 1992).

Intensive studies have been conducted on the effect of milk protein polymorphism on milk composition, which influences the renneting process. In goat milk, the ten CN alleles are associated with different amounts of α_{s1}-CN. Remeuf (1993) reported that the alleles are correlated with total N, casein and fat content. He also noted that micellar size (AA $<$ EE and FF) and micellar mineralization (FF and EE $>$ AA) were affected by α_{s1}-CN genetic variants. The same author postulated that considerable differences in the renneting speed, curd firmness and cheese yield may be attributable to the three groups of alleles. Best parameters were obtained by homozygous α_{s1}-CN AA goats. In relation to taste with alleles, allele A gives goat cheese the sweetest flavor, while allele F provides the sharpest taste (Vassal and Delacroix-Buchet 1994).

69.6 PHYSICOCHEMICAL PROPERTIES AND PROCESSING CHARACTERISTICS OF GOAT MILK

69.6.1 Physicochemical Properties and Processing Variables

Goat and cow milk fats have a similar level of the unsaponifiable matter (Table 69.13). However, goat milk fat contains more unsaturated fatty acids than the cow counterpart, because the former has higher iodine values than the latter. Saponification value is higher and refractive index is slightly higher in cow milk than in goat milk, whereby both indices reflect the number of carbons and saturation in the fatty acids in the milks. Most short chain acids (C4–C8) in goat milk triglycerides are esterified at position *sn*-3 of the glycerol, while the longer chains (C10 or greater) are at position *sn*-2 (Park 2006). Triglycerides are synthesized from a pool of long-chain 1,2-diglycerides (Tziboula-Clarke 2003). Goat milk fat contains more soluble volatile fatty acids and less insoluble volatile fatty acids than cow milk fat.

TABLE 69.13 Comparison of Physicochemical, Renneting Variables, and Micelle Structure of Goat Milk with Those of Cow Milk.

Characteristics	Goat Milk	Cow Milk
Physicochemical values[a]		
Unsaponifiable matter of milk fat (%)	0.41 ± 0.02	0.41 ± 0.02
Iodine value	30.44 ± 2.57	27.09 ± 1.26
Saponification value	228.6 ± 5.24	232.3 ± 7.61
Refractive index	1.450 ± 0.39	1.451 ± 0.35
Renneting variables[b]		
Renneting time (min)	19.6	35
Firmness of gel (mV)	43.9	75
Setting speed of gel (mV per min)	3.6	2
Volume of water retained in centrifuged curd	5.0	8.6
Micelle Structure[b]		
Noncentrifugal casein (% of total casein)	8.7	5.7
Average diameter (nm)	260	180
Hydration of micelle (g/g MS)	1.77	1.9
Mineralization of micelle (g/ca/100 casein)	3.6	2.9

Source: [a]Anjaneyulu and others (1985); [b]Remeuf and Lenoir (1986).

Goat milk has shorter renneting time than cow milk, whereas the firmness of goat milk gel is substantially less than that of cow milk (Table 69.13). The rennet setting speed of the gel for goat and cow milk are 3.6 and 2 mV/min, but the weak structure of goat gel causes some difficulties in manufacturing hard goat cheeses.

Goat milk has a different micelle structure compared to cow milk (Table 69.13). Caseinate micelles of goat milk contain more calcium and inorganic phosphorus, are less solvated, less heat stable, and lose β-CN more readily than bovine micelles (Jenness 1980). The average mineralization level in goat milk is higher than in cow milk (Table 69.13). However, the degree of hydration in goat milk is lower, resulting in an inverse relationship between the mineralization of the micelle and its hydration in goat milk (Soods and others 1979; Remeuf and Lenoir 1986).

Soluble casein content of goat milk is higher than in cow milk. Goat and cow milk have 10 and 1% soluble casein at 20°C, while 25 and 10% soluble casein at 5°C, respectively (Juàrez and Ramos 1986). Low storage temperatures have a marked influence on micellar system. Cooling leads to a partial solubilization of colloidal calcium phosphate and of β-CN (O'Connor and Fox 1973).

69.6.2 Heat Stability of Goat Milk

Heat stability of goat milk is considerably less than for bovine milk. High ionic calcium content and low micellular solvation in caprine milk may contribute to heat instability (Remeuf 1992). Goat milk has a great sensitivity toward heat treatment (Chandan and others 1968; Lavigne and others 1989), whereby it is usually unable to withstand UHT treatment (Patton and others 1980). However, recently goat milk is routinely processed by the UHT method in commercial goat dairies such as in the Wisconsin dairy goat cooperative. A great variability in heat stability exists between individual milk samples. Heat coagulation times at 140°C were between 0.5 and 23.4 min (Chandan and others 1968),

while heat coagulation temperatures of individual goat milk samples ranged from 118°C to more than 140°C (O'Connor and Fox 1973).

Several methods including pH adjustment, addition of a calcium sequestrant, and preheating of milk, were proposed to improve heat stability of goat milk and to sustain UHT treatments for goat milk (O'Connor and Fox 1973; Patton and others 1980). However, the problem of stability in high temperature treated goat milk has not been resolved due to rapid destabilization and flavor alteration of UHT processed fluid goat milk.

The heat coagulation time versus pH profile for caprine milk samples heated at 140°C typically displayed a pronounced heat stability maximum at about pH 6.9, with low heat stability at both low and high pH (Fox and Hynes 1976; Tziboula 1997). The difference in the pH threshold between goat and cow milks may cause the variability in heat instability of goat milk, where the pH threshold of goat milk is around 6.9 while that of cow milk is 6.5–6.6 (Patton and others 1980). Goat milk shows a lower heat stability at its original pH of 6.7 when compared to cow milk. The differences between micellar characteristics or salt equilibria of the two milks also might be attributable to the lower heat stability of goat milk. Tziboula (1997) demonstrated that the heat stability of goat milk is dependent on the casein genotype, with milks high in α_{s1}-CN having a lower heat stability than those with a low α_{s1}-CN content.

69.7 GOAT MILK PRODUCTS AND THEIR MANUFACTURING TECHNOLOGY

Many types of dairy products can be manufactured from goat milk, including fluid products (low fat, fortified, or flavored), fermented products such as cheese, buttermilk or yogurt, frozen products such as ice cream or frozen yogurt, or butter, condensed and dried products (Malven 1977; Kehagias and others 1986). However, cheese is traditionally the main goat commercial milk product produced and consumed in large quantities around the world. Significant volumes of fluid, evaporated and powdered goat milk products have been marketed in the United States and New Zealand for the past several decades. Although literatures have been available on goat milk cheeses, research data on other manufactured dairy goat products have been scarce (Malven 1977; Kehagias and others 1986). The basic compositions of dairy goat products are shown in Table 69.14.

69.7.1 Beverage Milk

Using conventional processing equipment, fluid goat milk may be processed by standardizing to 2% fat and 10.5% SNF and fortified with vitamins A and D, and pasteurizing for retail sale (Loewenstein and others 1980). The general processing procedure for fluid cow milk may be applied for goat fluid milk processing, which is shown in Figure 69.2. Condensed goat milk may be used for standardization, even though nonfat dry milk should be used if available.

There are at least four important requirements for Grade A quality goat milk. Those are: (1) safe to drink, (2) good flavor, (3) low spoilage bacteria and somatic cells, and (4) minimum composition (Loewenstein and others 1984). For safe milk, it must be free of pathogenic bacteria, antibiotic, insecticide, and herbicide compounds (Loewenstein and others 1984; Park 2006).

TABLE 69.14 Basic Nutrient Contents (%) of Commercial U.S. Goat Milk Products (Wet Basis).

	Total Solids		Protein		Fat		Carbohydrates		Ash	
Goat Milk Product	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Fluid milk										
Recent study[a]	11.3	0.05	2.92	0.09	3.40	0.10	4.15	0.13	0.79	0.01
USDA[b]	13.0	0.15	3.56	0.03	4.14	0.05	4.45	–	0.82	0.01
Evaporated milk										
Recent study[a]	20.85	0.05	6.11	0.33	6.75	0.05	6.56	0.53	1.43	0.10
USDA[c]	25.86	0.08	6.81	0.03	7.56	0.01	10.04	–	1.55	0.02
Powdered milk										
Recent study[a]	94.1	0.56	27.0	0.45	28.2	1.35	32.0	0.33	6.77	0.15
USDA[d]	97.5	0.13	26.3	0.18	26.9	0.25	38.4	–	6.08	0.09
Yogurt[e]										
Plain	11.5	2.56	3.99	0.12	2.25	0.13	4.49	0.56	0.82	0.02
Blueberry	17.7	2.34	3.37	0.13	1.18	0.17	12.6	2.72	0.86	0.09
Cheese[f]										
Soft										
Plain	40.2	6.81	18.9	5.26	22.5	4.37	–	–	1.74	0.97
Herb	40.9	2.11	17.3	2.26	21.8	2.13	–	–	1.60	0.61
Hard										
Cheddar	8.3	1.76	30.3	0.56	26.6	1.13	1.40	–	3.60	0.13
Blue	74.1	1.62	20.2	0.35	31.8	1.06	–	–	3.32	0.36

[a]Means of eight fluid milk (two brands, four different lots), 12 evaporated milk (two brands, six different lots), and 10 powdered milk (two brands, five different lots) samples, respectively. *Source*: Park (2000); [b]Data for fluid goat milk from *USDA Handbook No. 8-1* (Posati and Orr 1976); [c]Evaporated canned milk from *USDA Handbook No. 8-1* (Posati and Orr 1976); [d]Powdered whole canned milk from *USDA Handbook No. 8-1* (Posati and Orr 1976); [e]Park (1994a); [f]Park (1990).
$\bar{X}$ = mean; SD = standard deviation.

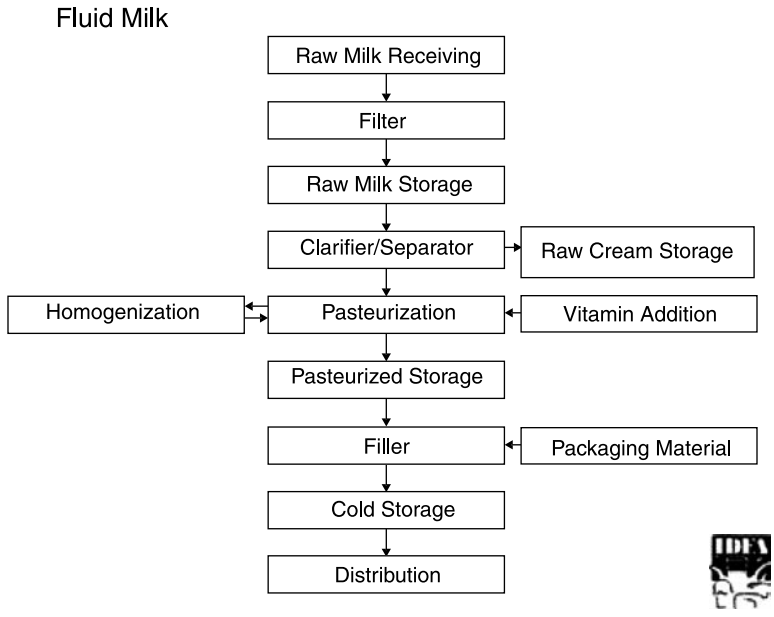

Figure 69.2 *General flowchart for commercial fluid milk processing procedures (Adapted from FDA Workshop, St. Louis, MO, 2000).*

69.7.2 Goat Milk Cheeses

Many different varieties of goat milk cheeses are produced worldwide, depending on diversity of locality, milk composition, and manufacturing techniques. Goat cheese composition would vary significantly between varieties due to the high variation in the seasonal composition of milk, modifications of manufacturing procedures, and multitude of aging time and conditions (Loewenstein and others 1980; Godina 1985; Park 1990). The varietal difference among cheeses is accounted for by the nature of physical and chemical changes during ripening (Fredriksen and Steinsholt 1978; Loewenstein and others 1984), which are affected by the cultures, chemicals, or flavor ingredients added to the curd during manufacturing processes (Lame and Hekmati 1975; Kosikowski 1977; Loewenstein and others 1980; Park 1990).

France has offered the best in goat milk cheeses, many of which are surface-ripened. France produces many exotic types of goat cheeses, including Crottin du Chavignol, Les Pyramides, Sainte Maure, Chabis and Chabicou (Kosikowski 1977). Other successful goat milk cheese producing countries are Norway, Spain, Greece, Portugal, Italy, and United States (Park and Guo 2006).

The Agricultural Handbook No. 54 of the USDA (Sanders 1969) describes over 400 varieties of goat cheeses and lists over 800 names of cheeses, made from goat milk or combinations of goat with other species milk such as cow, ewe, or buffalo (Park 1990).

Because the majority of goat cheeses are soft body types, the manufacture of goat cheese is referred to as a "cottage industry." A study on nutrient composition of U.S. goat cheeses showed that 20 out of 30 varieties investigated were very high or high moisture cheeses, suggesting that slow coagulation is the major mode of fabrication (Park 1990).

The traditional farmstead goat milk cheesemaking consists of the following nine basic steps (Le Jaouen 1987): (1) filtering of the milk, (2) pasteurizing, (3) renneting, sometimes preceded by acidification, (4) coagulation of the milk, (5) placing of the curds into cheese molds, sometimes preceded by predraining, (6) draining, sometimes interrupted by turning the cheeses over, (7) unmolding, (8) salting, (9) drying, and (10) ripening. These procedures are traditionally used for French soft body type farmstead goat cheese manufacture (Park and Guo 2006).

69.7.3 Goat Milk Yogurt

Goat milk yogurt is one of the traditional products from countries where fermented dairy foods originated (Park and Guo 2006). Goat milk yogurt can be made in a similar manner to the cow counterpart. The general manufacturing procedure for yogurt making is shown in Figure 69.3. One of the main problems in manufacture of goat milk yogurt is weak or lack of consistency in curd tension or viscosity upon agitation compared with cow yogurt. This is due in part to the difference in protein composition between the two milks, especially in casein contents.

The typical manufacturing steps for yogurt are shown in Figure 69.3 (Kosikowski 1977; Loewenstein and others 1980). The basic processing procedures of goat milk yogurt include: (1) preparation of milk, (2) standardization (standardized to 1.0–1.7% fat), (3) pasteurization (72°C for 20 s compared to cow yogurt 90.6°C for 40–60 s (HTST) or 85°C for 30 min (vat), (4) cool the pasteurized mix to 46.7°C (116°F) and hold in vat for up to 15 min, (5) inoculation (45°C) (Carefully introduce into warm milk or milk mixes 1.25% by weight of active *Lactobacillus bulgaricus* culture and 1.25% *Streptococcus thermophilus* culture), (6) packaging (set yogurt), (7) incubation (permit filled containers to remain in room at 45°C (114°F) for 3–5 h or until a firm, smooth gel has formed to

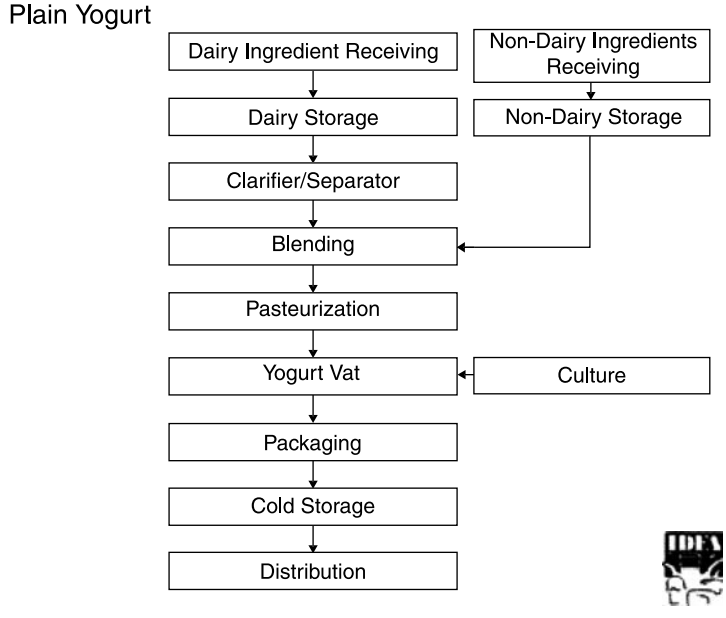

Figure 69.3 *Flowchart for manufacture of yogurt. (Adapted from FDA Workshop, St. Louis, MO, 2000.)*

pH 4.5), (8) chilling (yogurt is chilled to 7.2°C (45°F) in less than 1 h), and (9) storage and distribution (store the containers of yogurt at 4.4°C (40°F) or lower; the shelf life at this temperature is 30–60 days) (Kosikowski 1977; Park and Guo 2006).

69.7.4 Other Goat Milk Products

69.7.4.1 Other Fermented Goat Milk Products. Many other fermented goat milk products are produced and consumed in many parts of the world, such as buttermilk, acidophilus, sour dip, and kefir. Different culture microorganisms are used for these different types of fermented milk products, and incubation is stopped at different desired acidity (Park and Guo 2006).

Buttermilk is usually made from skim milk using the by-product from churning butter from sour cream. Goat buttermilk is made from skim milk (less than 0.5% fat); yogurt made from whole milk (3.25% fat), low fat milk (0.5–2.5% fat), or skim milk; sour cream must contain 18% fat in most states (Loewenstein and others 1980).

Acidophilus milk is pasteurized milk or low-fat milk inoculated with a probiotic *Lactobacillus acidophilus*, which destroys other competing bacteria antagonistic to man in the lower intestine. These organisms have the ability to implant themselves in the large intestines, survive the stomach acids and enzymes, low surface tension, and change nutrients (Kosikowski 1977). In the past the popularity of this product was limited by the flavor developed during fermentation. It is manufactured with skim milk or partially defatted milk, which is sterilized in an autoclave at 120°C for 20 min (15 psi), then tempered to 38°C. Next, a 5% inoculation of active *L. acidophilus* starter is introduced. The mixture is incubated at 38°C for 18 to 24 h until a curd forms with about 1.0% titratable acidity (Park and Guo 2006).

Kefir is an acidic, slightly foamy product, made from pasteurized and fat-standardized or decreamed goat milk, which has passed through a combined acidic and alcoholic fermentation of symbiotic lactic acid bacteria and yeast "kefir grains" (Kosikowski 1977). The finished product, kefir contains 0.6–0.8% lactic acid, 0.5–1.0% alcohol and carbon dioxide (Park and Guo 2006).

Ghee is an Indian (and Middle-East) clarified butterfat product which is manufactured by fermenting whole milk into curd and churning out butter, followed by heat clarification at 105–145°C (Kosikowski 1977; Chandan and others 1992). In the Middle East, casein is produced from skimmed milk. In Iran it is called *Kashk* or dried butter.

Sweet products made of goat milk are popular in Mexico, Norway, and India (Park and Guo 2006). In Mexico, the *Caheta* is a thick liquid of caramelized milk with sugar added, which is popular and sold as such or dried as small tarts. In Norway, *gjetost*, brown whey cheese, is produced by heat condensation and is similar in taste to *Cajeta*. *Gjetost* is a sweet caramel-color product with a firm texture in which lactose crystals may be noted. In India, a goat milk-based sweet is made by kneading chhana and cooking in sugar syrup over medium heat. *Khoa* is a heat-desiccated indigenous milk product used in the preparation of a variety of sweets (Park and Guo 2006).

Cosmetic goat milk products such as goat milk soap, hand lotion, and so on, have been produced and are increasingly popular in the United States and other countries like Switzerland. An internet search on goat milk soap shows a list of more than 5000 references. The number of home-based goat milk soap businesses has tremendously increased in recent years, and now generate substantially high annual revenues in the United States (Park and Guo 2006).

69.7.4.2 Evaporated and Powdered Goat Milk Products. Little research has been reported on evaporated and powdered goat milk products, while they are manufactured in the United States and New Zealand, marketed around the world (Park 2000; Park and Guo 2006). Evaporation is usually done under reduced pressure, primarily to allow boiling at a lower temperature and thus prevent flavor damage due to heating. The principal components of an evaporation plant are: (1) evaporation chambers operating as heat exchangers, (2) equipment for the production and maintenance of a vacuum, (3) separators for the separation of vapor and concentrate, and (4) a condenser for the vapor (Cross and Overby 1988; Park and Guo 2006). General composition of evaporated cow milk is 7.5–9.0% fat, 17.5–22% milk solids nonfat, and 25–31% total solids, while that of goat milk counterpart is shown in Table 69.14.

In manufacturing powdered milk, two different processing methods are used for dried milk products; roller drying, and spray drying process. In the roller drying process, milk or milk concentrate is applied in a thin film on the surface of a rotating, steam-heated metal drum (Park and Guo 2006). During the rotation the milk film dries and is continuously scraped off by a stationary knife located opposite the point of application of the concentrate. The spray drying process involves the transformation of fluid state into a dried particulate by spraying the milk into a hot drying medium. Four process stages of conventional spray drying include: (1) atomization of milk into a spray, (2) spray drying air contact (mixing and flow), (3) drying of spray (water evaporation), (4) separation of dried product from the air (Cross and Overby 1988).

69.7.4.3 Frozen Goat Milk Products. Goat milk ice cream can be manufactured, but little research has been documented (Loewenstein and others 1980). Three formulations made for three flavors of goat ice cream may be: (1) French vanilla mix with 14% fat, 10% MSNF, 18% sweetener (12% sucrose, 6% 36-dextrose equivalent corn syrup solids), 1.4% egg yolk solids, and 0.25% stabilizer-emulsifier; (2) chocolate mix: 14.6% fat (0.6% cocoa fat), 9% MSNF, 20% sweetener (14% sucrose, 6% 36-DE corn syrup solids), 3% medium fat cocoa, and 0.22% stabilizer-emulsifier; (3) premium white mix: 15% fat, 10% MSNF, 18% sweetener and 0.25% stabilizer-emulsifier (Park and Guo 2006).

69.8 ADVANTAGES OF GOAT MILK IN HUMAN NUTRITION AND HEALTH

Goat milk has been recommended as a substitute for cow milk for patients who suffer from allergies against cow milk or other food sources (Rosenblum and Rosenblum 1952; Walker 1965; Taitz and Armitage 1984; Van der Horst 1976; Park 1994b). Most patients who are allergic to cow milk proteins can tolerate goat milk well. Some caprine milk proteins have shown immunological cross-reaction with cow milk proteins, but infants suffering from gastrointestinal allergy and chronic enteropathy against cow milk were reportedly cured by goat milk therapy (Rosenblum and Rosenblum 1952; Walker 1965; Firer and others 1981; Park 1994b).

When compared to cow or human milk, many reports have shown that goat milk possesses unique characteristics, such as high digestibility, distinct alkalinity, high buffering capacity as well as certain therapeutic values in medicine and human nutrition (Gamble and others 1939; Walker 1965; Devendra and Burns 1970; Haenlein and Caccese 1984; Park and Chukwu 1988; Park 1991, 1994b).

Since goat milk contains high amounts of short chain and medium chain fatty acids (MCT) in its milk fat, goat milk may make at least three significant contributions to human nutrition (Haenlein 1981; Park and Haenlein 2006): (1) goat milk fat may be more rapidly digested than cow milk fat because lipase attacks ester linkages of short or medium chain fatty acids more easily than those of longer chains (Jenness 1980; Chandan and others 1992; Park 1994b), (2) these fatty acids have the unique metabolic ability to provide energy in growing children, and also exhibit beneficial effects on cholesterol metabolism such as hypocholesterolemic action on tissues and blood via inhibition of cholesterol deposition and dissolution of cholesterol in gallstones (Greenberger and Skillman 1969; Kalser 1971; Tantibhedhyangkul and Hashim 1975; Haenlein 1992), and (3) they also have been therapeutically used for treatment of various cases of malabsorption patients suffering from steatorrhea, chyluria, hyperlipoproteinemia, intestinal resection, coronary bypass, childhood epilepsy, premature infant feeding, cystic fibrosis, and gallstones (Greenberger and Skillman 1969; Tantibhedhyangkul and Hashim 1975; Haenlein 1992; Park 1994b).

Average diameters of the fat globule for goat, cow, buffalo and sheep milk were reported as 3.49, 4.55, 5.92, and 3.30 μm, respectively (Fahmi and others 1956; Juàrez and Ramos 1986). This smaller fat globule size of goat milk has an advantage of better digestibility compared to cow milk counterparts (Haenlein and Caccese 1984; Stark 1988; Chandan and others 1992; Park 1994b). Fat globule size of goat and other species milks may vary with breeds within species.

When fat in human milk is compared with that in cow milk, the former is absorbed more readily by infants than the latter (Grzesiak 1997). This is probably due to the difference in arrangement of fatty acids in the triglycerides (Jenness 1980; Heyman and others 1988). Due to the similarity of distribution of fatty acids over the positions in the triglycerides in goat and cow milks, the efficiencies of absorption of both milk fats are expected to be similar if fat globule size is not considered (Park 2006). Palmitic acid (C16 : 0) is shown to be primarily esterified in the two-position of the triglycerides in human milk fat, whereas the C16 : 0 acid is distributed nearly equally among the three positions in cow milk fat (Heyman and others 1988; Grzesiak 1997). If palmitic acid is located in the two-position, the digestive and absorptive processes are reportedly greatly enhanced (Park 2006).

The characteristics of goat milk proteins have also been shown to be advantageous, where they are digested more readily by stomach proteases. Their amino acids are absorbed more efficiently than those of cow milk, because goat milk caseins form a softer, more friable curd when acidified. These characteristics are related to differences in goat milk protein polymorphisms, especially lower contents of α_{s1}-casein, relative to cow milk (Jenness 1980; Haenlein and Caccese 1984; Juàrez and Ramos 1986; Chandan and others 1992).

Compared to cow milk, goat milk has been shown to have a better buffering capacity, which is good for the treatment of ulcers (Watson 1931; Park 1991, 1992a). The buffering capacity (BC) is influenced by proteins, primarily casein and phosphate systems in milk (Watson 1931). Nubian goat milk had highest levels of total N, protein, NPN and phosphate (P_2O_5) among the four milks of goat and cow breeds (Table 69.9). Because of the compositional differences, milk of the Nubian goat breed showed a higher BC compared with the milk of the Alpine goat breed, Holstein and Jersey cows (Park 1991). Major buffering entities of milks were influenced by species and breeds within species (Table 69.9). Regardless of breed, goat milk contained significantly higher NPN than cow milk. The higher levels of nitrogen moieties and phosphate in goat milk were positively correlated

with higher BC (Park 1991). Soy-based infant formulae contained less total N and NPN compared with natural goat and cow milks, and BC of the formulae were also lower than those of natural milks. This suggests that the higher BC in Nubian goat milk compared to cow milk can be of importance in human nutrition (Park and Haenlein 2004).

In a comparative growth trial (Mack 1953), children fed goat milk showed significantly greater nutrient bioavailability and growth parameters than those fed cow milk. In an iron bioavailability study of goat and cow milks using anemic rats, rats fed on goat milk had grown significantly better, had higher liver weights, hemoglobin iron gain, and higher iron absorption rates than those on cow milk (Park and others 1986). The anemic rats receiving the whole goat milk diet showed significantly greater hemoglobin regeneration efficiencies than those on the cow milk diet. On the other hand, goat milk has been blamed for the development of "goat milk anemia" due to the deficiency of folic acid in the milk (Fries 1959; Sandine and Daly 1979; Firer and others 1981; Perdue and others 1990; Haenlein 2004). Nevertheless, goat milk has been recommended as a nutritionally healthy and therapeutically functional food source for patients suffering from various allergies, as well as those who seek better nutrient absorption, greater nutrient bioavailability, and higher buffering capacity, and so on (Rosenblum and Rosenblum 1952; Walker 1965; Taitz and Armitage 1984; Park 1994b).

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70

Bioactive Peptides in Dairy Products

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70.1 Introduction 489
70.2 Opioid Peptides 491
70.3 Caseinophosphopeptides 493
70.4 Immunomodulatory Peptides 495
70.5 Cytomodulatory Peptides 495
70.6 Antithrombotic Peptides 496
70.7 Hypocholesterolemic peptides 496
70.8 ACE-Inhibitory (Antihypertensive) Peptides 497
70.9 Peptides Inhibitory to Bacterial Proteolytic Enzymes 503
70.10 Antimicrobial Peptides 503
70.11 Conclusions 510
References 510

70.1 INTRODUCTION

Fundamental studies have opened new fields of research dealing with bioactive or biogenic substances, including peptides, which are derived from foods. First, bioactive peptides (BPs) were mentioned by Mellander in 1950, who described the effect of casein (CN)-derived phosphorylated peptides in the vitamin D-independent bone calcification of rachitic infants. Thereafter, several definitions have been given for BPs. One of the

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Bioactive Agent: Milk	Bioactive Agent: Gut		Action
A. GI development, activity, and function			
Casein	casomorphins	↓	Gut motility, passage rate of digesta
		↓	Gastric emptying rate
		↑	Uptake of amino acids, electrolytes by intestinal epithelial cells
Casein	Casokinins (ACE-I)	↑	Blood flow to intestinal epithelium
Growth factors	IGF-1, TGF-α, EGF	↑	Growth in intestinal tract
Lactoferrin	Lactoferrin	↑	Proliferation of intestinal epithelial cells
B. Infant development			
Milk peptide	Milk peptide	↑	Lactotrope development in pituitary of suckling
Prolactin	Prolactin	↑	Lymphocyte trafficking, immune development
Growth factors	IGF-1, TGF-α, EGF, TGF-β	↑	Liver, organ development and function
PTHrP	PTHrP	↑	Ca^{2+} metabolism, uptake?
Prosaposin	Prosaposin?	↑	Neurological development?
C. Immunological development and function			
Immunoglobulins	IgG, IgA	↑	Passive immunity
Cytokines	Interleukins-1,-2,-6,-10; Tumor necrosis factor-α; Interferon-γ, Transforming growth factors-α,-β; Prostaglandins E_2, F_{II}; leukotriene B_4	↑	Lymphocyte trafficking, immune development
Prolactin	Prolactin	↑	Thymocyte trafficking, immunological development
Lactoferrin	Lactoferrin	↑	Humoral immune response to sheep RBC
		↑	T-helper cell ($CD4^+$) development
		↑	Natural killer cell activity
		↑	Lymphokine-activated killer cell activity
		↓	TFN-α
		↑	IL-6
Lactoferrin	Lf N-terminal peptides + Lactoferricin	↑	Humoral immune response
		↓	Inflammatory response to bacterial endotoxin
Caseins	Casomorphins Casokinins	↑	Immune response, phagocytic activity
D. Microbial activity			
a. Antibiotic activity – *Antibacterial, antiviral activity, non-immune disease defense:*			
Immunoglobulins	IgG, IgA	↑	Passive immunity to bacterial and virus disease
α_{S1}- Casein	Isracidin	↑	Bactericidal killing of several microorganisms
κ- /α_{S1}- Casein	Casecidins	↑	Bactericidal killing of several pathogenic strains of *Staphylococcus aureus* and lactobacilli
α_{S2}- Casein	Casocidins-I	↑	Bactericidal killing of *Staphylococcus carnosus,Escherichia coli*
κ- Casein	Kappacin	↑	Bactericidal killing of *Streptococcus mutans, Porphyromonas gingivalis, Escherichia coli*
Lactoferrin	Lactoferrin	↑	Bacteriostatic inhibition of iron-dependent bacteria
		↓	Viral attachment to and infections of cells
		↑	Lymphokine-activated killer cell activity
Lactoferrin	Lf+, Lactoferricin	↑	Bactericidal killing of Gram +/– enteropathogens
Glycolipids, Oligosaccharides	Glycolipids, Oligosaccharides	↓	Bacterial and viral attachment to intestinal epithelial cells (decoy receptors)
		↓	Bacterial colonization and viral infection
b. Probiotic activity – *Growth of Bifidobacteria and Lactobacilli in GI tract*			
Lactoferrin	Lactoferrin	↑	Growth of Bifidobacteria in GI tract
κ-Casein	κ-Casein	↑	Growth of Bifidobacteria in GI tract
Oligosaccharides	Oligosaccharides	↑	Growth of Bifidobacteria in GI tract

An arrow pointing down (↓) means a decrease of the biological action, whereas an arrow pointing up (↑) means an increase.

Figure 70.1 *Major categories of bioactivities in milk (A and others E), and their general effects and major effectors. Adapted from Schanbacher 1998.*

most appropriate is the following: components (genuine or generated) of consumption ready foods which may exert a regulatory activity in the human organism, irrespective of their nutritive functions (Meisel 2001). Bioactive substances of food origin could be delivered to the consumer in conventional foods, dietary supplements, functional foods, or medical foods whereby each category requires specific claims. In 1991 Japan has defined the concept of Food for Specified Health Use (FOSHU) and in 1993 the United States introduced health claims for foods having the properties of reducing diseases.

Recently, some guidelines for the manufacture of functional foods have been introduced in Europe also (Martin 2000).

Milk proteins are currently the main source of a range of biologically active peptides (Pihlanto-Leppälä 2001). Milk is known to contain an array of bioactivities which extends the range of influence of mother over young beyond nutrition alone. Several of these bioactivities are attributable to proteins and peptides secreted into milk by the mammary gland. (Fig. 70.1). The major categories of bioactivities in milk, and their general effects and major effectors are summarized in Figure 70.1. Four major bioactivity categories may be distinguished: (1) gastrointestinal development, activity, and function; (2) infant development; (3) immunological development and function; and (4) microbial activity, including antibiotic and probiotic actions.

Nevertheless, most of the bioactivities of milk proteins are latent, being absent or incomplete in the native protein and only fully manifested upon proteolytic digestion to release and activate an encrypted BP from within (Gobbetti and others 2002). Proteolysis may release BPs during gastrointestinal transit or during food processing. Digestive enzymes, naturally occurring enzymes in milk, coagulants and microbial enzymes, especially those from adventitious or starter lactic acid bacteria, generate BPs during milk fermentation and cheese maturation, thereby enriching the dairy products (Gobbetti and others 2002). Interestingly, several milk-derived peptides reveal multifunctional properties, that is, specific peptide sequences having two or more different physiological activities. Some regions in the primary structure of CN contain overlapping peptide sequences that exert different activities. These regions have been considered as strategic zones that are partially protected from further proteolytic breakdown (Fiat and others 1993).

The major peptide bioactivities in dairy products, their synthesis through food processing and their putative or *in vivo* physiological role are described in the following sections.

70.2 OPIOID PEPTIDES

Opioids are defined as peptides (e.g., enkephalins) which have an affinity for an opiate receptor and opiate-like effects, inhibited by naloxone. Milk protein-derived BPs may function as regulatory substances, defined exorphins, with pharmacological properties similar to enkephalins (Meisel and others 1989; Meisel and Schlimme 1990; Schanbacher and others 1998) (Table 70.1). The α_{S1}-casein-exorphin (α_{s1}-CN f90–96), β-casomorphins-7 and -5 (β-CN f60–66 and f60–64, respectively), and lactorphins (α-lactalbumin f50–53 and β-lactoglobulin f102–105) act as opioid agonists, while casoxins (e.g., κ-CN f35–42, f58–61, and f25–34) act as opioid antagonists (Meisel and FitzGerald 2000). An anticonvulsant and anxiolytic molecule, α-casozepine, not structurally related to benziodiazepines, was produced by trypsin hydrolysis of bovine α_{s1}-CN. It corresponded to α_{s1}-CN f91–100. As shown in conditioned defensive burying rat models, this peptide might play a role, as external ligand, in the regulation of the nervous system of the mammalian newborn (Miclo and others 2001).

The common structural feature among endogenous and exogenous opioid peptides is the presence of a Tyr residue at the amino terminal end (except for α_{S1}-casein-exorphin, casoxin 6, and lactoferroxin B and C) and of another aromatic residue, Phe or Tyr, in third or fourth position. The negative potential, localized in the vicinity of the phenolic hydroxyl group of Tyr, seems to be essential for opioid activity. Removal of the Tyr residue results in a total absence of activity (Chang and others 1981). The Pro residue

TABLE 70.1 Examples of Opioid Peptides Derived from Milk Proteins.

Bioactive Peptide	Sequence[1]	Precursor Protein (Fragment)[2]	IC_{50}^{3} (μmol/L)	Theoretical Yield of Bioactive Peptide Obtainable from 1 g Precursor Protein	References
Opioid agonists					
β-Casomorphin-7	YPFPGPI	β-CN f(60–66)	14.0	33.0	Brantl and others 1981
β-Casomorphin-5	YPFPG	β-CN f(60–64)	1.1	24.2	Meisel 1997
β-Casomorphin-11	YPFPGPIPNSL	β-CN f(60–70)	—	—	Meisel 1986
α_{S1}-Casein exorphin	RYLGYLE	α_{S1}-CN f(90–96)	1.2	38.7	Loukas and others 1983
α-Lactorfin	YGLF	α-lactalbumin f(50–53)	67.0	35.2	Chiba and Yoshikawa 1986
β-Lactorfin	YLLF	β-lactoglobulin f(102–105)	38.0	30.2	Chiba and Yoshikawa 1986
Serorphin	YGFQNA	Serum albumin f(399–404)	85.0	10.5	Tani and others 1994
Opioid antagonists					
Casoxin A	YPSYGLNY	κ-CN f(35–42)	400.0	51.4	Yoshikawa and others 1986
Casoxin B	YPYY	κ-CN f(58–61)	—	31.8	Chiba and others 1989
Casoxin C	YIPIQYVLSR	κ-CN f(25–34)	50.0	65.8	Yoshikawa and others 1986
Casoxin D	YVPFPPF	Human α_{S1}-CN f(158–164)	—	—	Yoshikawa and others 1994
Casoxin 6	SRYPSY(OCH_3)	κ-CN f(33–38)	—	—	Chiba and others 1989
Lactoferroxin A	YLGSGY (OCH_3)	Human lactoferrin f(318–323)	—	—	Tani and others 1990
Lactoferroxin B	RYYGY	Human lactoferrin f(536–540)	—	—	Tani and others 1990
Lactoferroxin C	KYLGPQY	Human lactoferrin f(673–679)	—	—	Tani and others 1990
Benzodiazepine-like activity					
α-Casozepine	TSKEDAMKAYID KVEELKKKYGI	α_{S1}-CN f(91–100)	—	—	Miclo and others 2001

Source: Meisel and Fitzgerald (2000).

[1]The one-letter amino acids codes were used; Phosphoserine = S*.

[2]Unless otherwise indicated, the precursor protein is from bovine milk.

[3]In relation to opioid peptides, peptide concentration required to inhibit 3H-ligand binding by 50%.

in the second position is also crucial to maintain the proper orientation of the Tyr and Phe side chains (Mierke and others 1990).

Modulation of social behavior and analgesic effects were found following intracerebral administration of opioid peptides with agonistic activity, for example, β-casomorphins, to experimental animals. Orally administered milk protein-derived opioid peptides have been demonstrated to influence postprandial metabolism by stimulating secretion of insulin and somatostatin, to modulate intestinal transport of amino acids, to prolong gastrointestinal transit time and to exert antidiarrhoeal action (Meisel and Schlimme 1990).

Evidence for the *in vivo* liberation of β-casomorphins from β-CN was found in the small intestine of adult humans following cow's milk intake (Svedberg and others 1985), in the duodenal chime of Göttingen minipgs after feeding with bovine CN (Meisel 1986), in serum of newborn calves after milk intakes (Umbach and others 1985), and in plasma of pregnant or breastfeeding women (Koch and others 1988). β-Casomorphins were found in the analogous position of the native protein in cow, sheep, water buffalo, and human β-CN (Meisel and Schlimme 1996). A number of caseolytic bacterial species used in the manufacture of cheeses and other milk products may synthesize casomorphins (Hamel and others 1985). Several opioid peptides derived from α_{s1}- and β-CN, and α-lactalbumin were released by pepsin/trypsin hydrolysis of *Lactobacillus* GG fermented UHT milk (Rokka and others 1997). Proteolysis of α-lactalbumin with pepsin produced directly α-lactorphin, while digestion of β-lactoglobulin with pepsin and then trypsin yielded β-lactorphin. Contrarily to β-casomorphins, there is no current evidence for the liberation of lactorphins during gastrointestinal digestion (Pihlanto-Leppälä 2001).

70.3 CASEINOPHOSPHOPEPTIDES

Caseinophosphopeptides (CPPs) or mineral binding phosphopeptides may form soluble organophosphate salts and function as carriers for different minerals, especially Ca^{2+} (Meisel and Olieman 1998). It is thought that CPPs synthesized during CN digestion enhance the biodisponibility of Ca^{2+} in the diet by increasing the solubility of Ca^{2+} in the alkaline environment of the small intestine, where the maximal passive absorption of dietary Ca^{2+} takes place (FitzGerald 1998).

CPPs may derive from different phosphorylated regions of α_{s1}-, α_{s2}-, β-, and κ-CN (Fig. 70.2). Most CPPs contain a common motif, for example, a sequence of three phosphoseryl followed by two glutamic acid residues. The negatively charged side chains, in particular the phosphate groups, of these amino acids represent the binding sites for minerals. Dephosphorylated peptides do not bind minerals (Berrocal and others 1989) or chemical phosphorylation of α_{s1}- and β-CN increased the binding capacity and the stability of these proteins in the presence of Ca^{2+} (Yoshikawa and others 1981). While the anionic hydrophilic domain is important in mineral binding, the amino acid residues upstream and downstream from this region play a role in binding also. Approximately one mole of CPP could bind 40 moles of Ca^{2+}. The Ca^{2+} binding constants of CPPs are in the order of 10^2–10^3 M^{-1} (Sato and others 1983; Schlimme and Meisel 1995). Whey proteins such as α-lactalbumin, β-lactoglobulin, and lactoferrin may also give rise to peptides with mineral binding abilities (Vegarud and others 2000). Since these peptides are not phosphorylated, the binding sites for minerals may be dependent on the particular conformation.

α_{S1}-casein	
DIGS*ES*TEDQAMEDIM	f(43–58) (Meisel and Olieman 1998)
QMEAES*IS*S*S*EEIVPNS*VEQK	f(59–79) (Schlimme and Meisel 1995)
S*S*S*EEIVPN	f(66–74) (Meisel and Frister 1988)
VPNS*AEER	f(112–119)
α_{S2}-casein	
KNTMEHVS*S*S*EES*IIS QETYKQEQNMAINPSK	f(1–32) (Hata and others 1998; Hata and others 1999)
EHVSS*S*EESIIS*QE	f(5–18)
NPS*KEN	f(29–34)
GS*S*S*EES*AEV	f(55–64)
QLS*TS*EENSKKTVDMES*TEVF	f(127–147)
β-casein	
RELEELNVPGEIVES*LS*S*S*EESITR	f(1–25) (Meisel and Olieman 1998)
IVES*LS*S*S*EESI	variant A f(12–23)
IVES*LS*KS*EESI	variant D f(12–23)
NVPGEIVES*LS*S*	f(7–18) (Chabance and others 1998)
KIEKFQS*EEQQQT	f(29–41) (Chabance and others 1998)
VYPFPGPIPNS*LPQNIPPLTQ	f(59–79) (Hata and others 1998; Hata and others 1999)
κ-casein	
EAS*PEVI	f(147–153)

Figure 70.2 *Sequence of different phosphorylated casein regions found in bovine milk proteins (The one letter amino acid codes were used; S* = serine phosphate). Adapted from Fitzgerald 1998.*

CPPs are generated from CN by enzymatic digestion with pancreatic endoproteinases, especially trypsin. Other enzyme combinations, including chymotryipsin, pancreatin, papain, pepsin, thermolysin, and pronase, have been used for *in vitro* CPPs production (FitzGerald 1998). Proteolytic enzymes from a range of bacterial and fungal sources have been used also (McDonagh and FitzGerald 1998). CPPs were synthesized upon hydrolysis of six different types of sodium-caseinate by a partially purified proteinase of *Lactobacillus helveticus* (Corsetti and others 2003). CPPs are formed *in vivo* following digestion of CN by gastrointestinal proteinases (Naito and others 1972; Sato and others 1983; Meisel and Frister 1988). CPPs are relatively resistant to further proteolytic degradation (Hirayama and others 1992; Kasai and others 1995) and can accumulate in the distal ileum (Naito and others 1972; Meisel and Frister 1988). A number of *in vivo* feeding trials involving Ca^{2+} balance studies after administration of CPPs have failed to show a significant effect on the absorption of Ca^{2+} in rats, piglets, minipgs and vitamin D-deficient rats (FitzGerald 1998). Nevertheless, a 23% improvement in intestinal soluble Ca^{2+} was reported from *in vivo* rat studies when comparing conventional CPPs to β-CN derived CPPs (Han and others 1996). Very little information is available from human studies. CPPs increase Ca^{2+} and Zn^{2+} absorption from a rice-based infant gruel in human

adults by approximately 30%. No effect was seen when CPPs were ingested in either high or low phytate wholegrain cereal meals (Hansen 1995).

Although the main current applications of CPPs center on their ability to solubilize minerals, an anticariogenic mechanism has been attributed also. It is related to the ability of localising high level of amorphous Ca^{2+} phosphate at the tooth surface and, as a consequence, to depress demineralization and enhance remineralization of tooth enamel (Reynolds 1994). CPPs may also act as buffering agents against plaque acid. Based on these properties, the opportunity for the incorporation of CPPs into dental hygiene care products has been created (FitzGerald 1998).

70.4 IMMUNOMODULATORY PEPTIDES

Although the mechanisms are not currently elucidated, milk-derived peptides have shown immunomodulatory activity. α_{s1}-CN f194–199 (α_{s1}-immunocasokinin) and β-CN f193–202, f63–68, and f191–193 (immunopeptides), synthesized by hydrolysis with pepsin-chymosin, stimulated phagocytosis of sheep red blood cells by murine peritoneal macrophages and exerted a protective effect against *Klebsiella pneumoniae* infection in mice after intravenous administration (Gill and others 2000). β-Casomorphin-7 inhibited the proliferation of human colonic lamina propria lymphocytes where the antiproliferative effect of micromolar concentrations was reversed by the opiate receptor antagonist naloxone (Elitsur and Luk 1991). β-Casomorphin-7 and β-CN immunopeptides suppressed the proliferation of human peripheral blood lymphocytes at low concentrations ($<10^{-7}$ mol/L) but stimulated it at higher concentration (Kayser and Meisel 1996). Immunoenhancing properties have been attributed to CPPs also (Hata and others 1999). Several β-CN derived peptides enhanced the immunoglobulin IgG production in mouse spleen cell cultures.

The results obtained with human lymphocytes suggested that opioid peptides may affect the immunoreactivity of lymphocytes via the opiate receptor. There is indeed a remarkable relationship between the immune system and opioid peptides, because opioid μ receptors for endorphins are present in lymphocytes (Elitsur and Luk 1991). It is worth noting that glutamine-containing peptides may substitute for the free amino acid glutamine which is required for lymphocyte proliferation and utilized at a high rate by immunocompetent cells (Calder 1994). It is conceivable that such peptides exert a non-specific immunostimulation as a result of their trophic properties.

70.5 CYTOMODULATORY PEPTIDES

Based on various cytochemical studies, there is an increasing evidence of the possible involvement of milk-derived peptides as specific signals that may trigger viability of cancer cells. Bacterial hydrolysis of CN using commercial yogurt starter cultures yielded bioactive peptides that influence colon cell Caco-2 kinetics *in vitro* (MacDonald and others 1994). Bovine skimmed milk digested with cell-free extract of the yeast *Saccharomyces cerevisiae* was found to have antiproliferative activity towards leukaemia cells (Roy and others 1999). Purified peptides, corresponding to bioactive sequences of CN, revealed modulation of cell viability, for example, proliferation and apoptosis, in different human cell culture models (Hartmann and others 2000). Accordingly, apoptosis

of human leukaemia cells (HL-60) was induced by the opioide peptide β-casomorphin-7. Peptides from a lyophilized extract of Gouda cheese inhibited proliferation of leukaemia cells, even at low concentrations of 1 pmol/L (Meisel and Günther 1998). The antiproliferative effect of Gouda extract was shown to be the result of peptide-induced apoptosis. Cancer cell lines were more reactive to peptide-induced apoptotic stimulation than non-malignant cells.

70.6 ANTITHROMBOTIC PEPTIDES

The mechanism involved in milk clotting through interaction of κ-CN with chymosin and blood clotting processes, defined by the interaction of fibrinogen with thrombin, are comparable. In this regard, the C-terminal dodecapeptide of human fibrinogen γ-chain (residues 400–411) and the undecapeptide (residues 106–116) from bovine κ-CN are structurally and functionally quite similar (Clare and Swaisgood 2000). This CN-derived peptide, termed casoplatelin, affected platelet function and inhibited both the aggregation of ADP-activated platelets and the binding of human fibrinogen γ-chain to its receptor region on the platelets surface (Jollès and others 1986). A smaller κ-CN fragment (residues 106–110), casopiastrin, was synthesized by trypsin hydrolysis and exhibited antithrombotic activity by inhibiting fibrinogen binding (Jollès and Henschen 1982; Jollès and others 1986; Mazoyer and others 1992). A second segment of the κ-CN fragment, residues 103–111, inhibited platelet aggregation but did not affect fibrinogen binding to the platelet receptor (Fiat and Jollès 1989; Fiat and others 1993; Jollès and others 1986). Antithrombotic peptides have also been derived from κ-caseinoglycopeptides that were isolated from several animal species. Bovine κ-caseinoglycopeptide, the C-terminal end of κ-CN (residues 106–169), inhibited von Willebrand factor-dependent platelet aggregation (Chabance and others 1998). Two antithrombotic peptides, derived from human and bovine κ-caseinoglycopeptides, were identified in the plasma of 5-day-old newborns after breast-feeding and ingestions of cow's milk based formula, respectively (Chabance and others 1998). The κ-caseinoglycopeptide (106–171) of sheep CN decreased thrombin- and collagen-induced platelet aggregation in a dose dependent manner (Qian and others 1995).

70.7 HYPOCHOLESTEROLEMIC PEPTIDES

A tryptic hydrolysate of β-lactoglobulin showed hypocolesterolemic activity in rats (Nagaoka and others 2001). The serum cholesterol-lowering activity depends on the degree of fecal steroid excretion (Nagata and others 1982) which was high in rats fed with the hydrolysate. Studies on the hypocholesterolemic effects of proteins supported the hypothesis that a peptide with high bile acid-binding capacity could inhibit the reabsorption of bile acid in the ileum and decrease the blood cholesterol level (Iwami and othres 1986). The β-lactoglobulin tryptic hydrolysate also directly inhibited the *in vitro* absorption of micellar cholesterol in Caco-2 cells. Cholesterol is rendered soluble in bile salt-mixed micelles and then absorbed (Wilson and Rudel 1994). The micellar solubility of cholesterol in the presence of β-lactoglobulin tryptic hydrolysate was markedly low. Four bioactive peptides were identified in the hydrolysate which corresponded to β-lactoglobulin f9–14, f41–60, f71–75, and f142–146. Fragment 71–75, which had no peptic or tryptic digestive site, was chosen for animal studies, and it showed a

greater hypocholesterolemic activity than the drug β-sitosterol (Nagaoka and others 2001).

70.8 ACE-INHIBITORY (ANTIHYPERTENSIVE) PEPTIDES

Angiotensin-I converting enzyme (ACE, peptidyl-peptide hydrolase; EC 3.4.15.1) is a multifunctional ectoenzyme that is located in different tissues (e.g., plasma, lung, kidney, heart, skeletal muscle, pancreas, arteries, brain) and plays a key physiological role in the renin-angiotensin, kallikrein-kinin, and immune systems. This enzyme is responsible for the increase in blood pressure by converting angiotensin-I to the potent vasoconstrictor, angiotensin-II, and by degrading bradykinin, a vasodilatatory peptide, and enkephalins (Petrillo and Ondetti 1982). Therefore, ACE inhibition mainly results in a hypotensive effect but may also influence different regulatory systems involved in immuno-defense and nervous system activity (Meisel 1993). Recently, it has been demonstrated that treatment with brain-penetrating ACE inhibitors could slow the rate of cognitive decline in mild to moderate Alzheimer disease patients in comparison with other antihypertensive drugs (Ohrui and others 2004). The mechanism might be due either to the direct effects of brain-penetrating ACE inhibitors on renin-angiotensin system in the brain or to an increased level of brain substance P by ACE inhibitors. Increased substance P may augment the activity of neprilysin, a major amyloid-β peptide degrading enzyme in the brain, and thus may favourably influence the course of Alzheimer disease.

Antihypertensive are the most studied BPs. Exogenous ACE inhibitors having an antihypertensive effect *in vivo* were first discovered in snake venom (Ondetti and others 1977). Several ACE-inhibitory peptides were identified by *in vitro* enzymatic digestion of milk proteins or chemical synthesis of peptide analogs (Table 70.2) (Gobbetti and others 2002, 2004). ACE-inhibitors derived from milk proteins represent different fragments of CN, named casokinins (Meisel and Schlimme 1994), or whey proteins, named lactokinins (FitzGerald and Meisel 2000).

Structure-activity correlation among different peptide inhibitors of ACE indicate that binding to ACE is strongly influenced by the C-terminal tripeptide sequence of the substrate. ACE appears to prefer substrates or competitive inhibitors which mainly have hydrophobic (aromatic or branched side chains) amino acid residues at the three C-terminal positions. ACE inhibition studies with Trp, Tyr, Phe, or Pro residues were most effective in enhancing substrate binding (Cheung and others 1980). Nevertheless, the structure-activity relationship of ACE-inhibitory peptides has not yet been clearly established and very different hypotensive sequences have been derived from a large number of food proteins (Meisel 1997). It is postulated that the mechanism of ACE inhibition involves inhibitor interaction with an anionic binding site that is distinct from the catalytic site of the enzyme. Given the above, it is expected that peptide conformation, for example, the structure adopted in a specific environment, should contribute to ACE inhibitor potency (FitzGerald and Meisel 2000).

A number of *in vivo* and *in vitro* studies have demonstrated the antihypertensive effect of CN-derived peptides contained in dairy products (FitzGerald and Meisel 2000; Gobbetti and others 2004). Nakamura and others (1995) purified ACE-inhibitors from Calpis, a Japanese soft drink made from skim milk fermented by *Lactobacillus helveticus* and *S. cerevisiae*. The hypotensive peptides, Val-Pro-Pro and Ile-Pro-Pro, which originated from both α_{s1}- and β-CN, have been found in milk inoculated with *Lb. helveticus* (Yamamoto and

TABLE 70.2 ACE-Inhibitory Peptides Derived from Milk Proteins.

Name	Fragment[2]	Sequence[1]	Enzyme or Origin	References
Unnamed	α_{S1}-CN f(1–9)	RPKHPIKHQ	Gouda cheese	Saito and others 2000
α_{S1}-Casokinin	α_{S1}-CN f(23–34)	FFVAPFPEVFGK	Trypsin (+peptidase)	Maruyama and others 1985
α_{S1}-Casokinin-5	α_{S1}-CN f(23–27)	FFVAP	Proline endopeptidase	Maruyama and others 1985
α_{S1}-Casokinin	α_{S1}-CN f(25–27)	VAP	Synthetic	Maruyama and others 1987a
Unnamed	α_{S1}-CN f(24–27)	FVAP	Synthetic	Maruyama and others 1987a
Unnamed	α_{S1}-CN f(28–34)	FPEVFGK	Proline endopeptidase	Maruyama and others 1987a
Unnamed	α_{S1}-CN f(32–34)	FGK	Synthetic	Maruyama and others 1987a
Unnamed	α_{S1}-CN f(24–47)	FVAPFPEVFGKEKVNEL SKDIGSE	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	α_{S1}-CN f(104–109)	YKVPQL	*Lactobacillus helveticus* CP790 protease	Maeno and others 1996
α_{S1}-Casokinin	α_{S1}-CN f(142–147)	LAYFYP	Fermentation	Pihlanto-Leppälä and others 1998
Unnamed	α_{S1}-CN f(143–148)	AYFYPE	*Lactobacillus helveticus* CP790 protease	Yamamoto and others 1994b
Unnamed	α_{S1}-CN f(157–164)	DAYPSGAW	Fermentation	Pihlanto-Leppälä and others 1998
α_{S1}-Immunocasokinin	α_{S1}-CN f(194–199)	TTMPLW	Trypsin	Maruyama and others 1987b
Unnamed	α_{S1}-CN f(197–199)	PLW	Synthetic	Maruyama and others 1987b
Unnamed	α_{S1}-CN f(198–199)	LW	Synthetic	Maruyama and others 1987b
Unnamed	α_{S2}-CN f(25–32)	NMAINPSK	Trypsin	Tauzin and others 2002
Unnamed	α_{S2}-CN f(81–89)	ALNEINQFY	Trypsin	Tauzin and others 2002
Unnamed	α_{S2}-CN f(81–91)	ALNEINQFYQK	Trypsin	Tauzin and others 2002
Unnamed	α_{S2}-CN f(92–98)	FPQYLQY	Trypsin	Tauzin and others 2002
Unnamed	α_{S2}-CN f(174–179)	FALPQY	Trypsin	Tauzin and others 2002
Unnamed	α_{S2}-CN f(174–181)	FALPQYLK	Trypsin	Tauzin and others 2002
Unnamed	α_{S2}-CN f(182–184)	TVY	Trypsin	Tauzin and others 2002
Unnamed	Sheep α_{S1}-CN f(1–6)	RPKHPI	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	Sheep α_{S1}-CN f(4–8)	HPIKH	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	Sheep α_{S2}-CN f(4–8)	TVDK	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003

Unnamed	Goat α_{S2}-CN f(4–8)	HPIKH	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
β-Casomorphin-7	β-CN f(60–66)	YPFPGPI	pepsin	Brantl and others 1981; Kayser and Meisel 1996; Meisel and Günther 1998
β-Casokinin	β-CN f(84–86)	VPP	Fermentation (sour milk)	Nakamura and others 1995; Seppo and others 2003
Unnamed	β-CN f(73–82)	NIPPLTQTPV	Fermented milk	Gobbetti and others 2000
Unnamed	β-CN f(169–174)	KVLPVP	*Lactobacillus helveticus* CP790 protease and synthetic	Maeno and others 1996
Unnamed	β-CN f(47–52)	DKIHPF	Fermented milk	Gobbetti and others 2000
Unnamed	β-CN f(57–64)	SLVLPVPE	*Lactobacillus helveticus* CP790 protease	Yamamoto and others 1994b
Unnamed	β-CN f(43–69)	DELQDKIHPFATQ SLVYPFPGPIHNS	*Lactobacillus helveticus* CP790 protease	Yamamoto and others 1994b
Unnamed	β-CN f(58–72)	LVYPFPGPIPNSLPQ	Cheeses (Crescenza, Cheddar, Jarlsberg, Norvegia, blue cheeses)	Smacchi and Gobbetti 1998; Stepaniak and others 2001
Unnamed	β-CN f(60–68)	YPFPGPIPN	Gouda cheese	Saito and others 2000
Unnamed	β-CN f(108–113)	EMPFPK	Fermentation	Pihlanto-Leppälä and others 1998
Unnamed	β-CN f(177–179)	AVP	Synthetic	Maruyama and others 1987a
Unnamed	β-CN f(177–181)	AVPYP	Synthetic	Maruyama and others 1987a
Unnamed	β-CN f(179–181)	PYP	Synthetic	Maruyama and others 1987a
Unnamed	β-CN f(181–183)	PQR	Trypsin	Maruyama and others 1987a
Unnamed	β-CN f(193–198)	YQQPVL	Fermentation	Pihlanto-Leppälä and others 1998
Unnamed	Sheep β-CN f(199–204)	VRGPFP	Manchego cheese	Gòmez-Ruiz and others 2002
Unnamed	β-CN f(58–76)	LVYPFPGPIPNSLPQNIPP	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	Goat β-CN f(58–65)	LVYPFPGP	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	Buffalo β-CN f(58–66)	LVYPFPGPI	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003

(*Continued*)

TABLE 70.2 *Continued.*

Name	Fragment[2]	Sequence[1]	Enzyme or Origin	References
Unnamed	Human β-CN f(44–46)	QPQ	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	Human β-CN f(77–79) or f(137–139) or f(155–157)	VPQ	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	Human β-CN f(141–143) or f(163–165); κ-CN f(74–76)	IPQ	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	κ-CN f(25–34)	YIPIQYVLSR	Synthetic	Chiba and Yoshikawa 1991
Unnamed	κ-CN f(35–41)	YPSYGLNY	Synthetic	Chiba and Yoshikawa 1991
Unnamed	κ-CN f(58–59), α_{S1}-CN f(146–147) and f(159–160); β-CN f(114–115);	YP	Fermentation and synthetic	Yamamoto and others 1999
Unnamed	γ-CN f(108–113)	EMPFPK	Trypsin	Perpetuo and others 2003
Unnamed	γ-CN f(114–121)	YPVEPFTE	Trypsin	Perpetuo and others 2003
Unnamed	κ-CN f(108–110)	IPP	Fermentation (sour milk)	Nakamura and others 1995
α-Lactorphin	α-Lactalbumin f(50–53)	YGLF	Synthetic	Chiba and Yoshikawa 1986; Mullally and others 1996
Unnamed	α-Lactalbumin f(50–51), f(18–19)	YG	Synthetic	Kayser and Meisel 1996; Mullally and others 1996
β-Lactorphin	β-Lactoglobulin f(102–105)	YLLF	Synthetic	Chiba and Yoshikawa 1986; Mullally and others 1996
Lactokinin	β-Lactoglobulin f(102–103)	YL	Synthetic	Mullally and others 1996
Lactokinin	β-Lactoglobulin f(142–148)	ALPMHIR	Trypsin	Mullally and others 1997
Serokinin	Serum albumin f(208–216)	ALKAWSVAR	Synthetic	Chiba and Yoshikawa 1991
Unnamed	Goat β-lactoglobulin f(58–61)	LQKW	Thermolysin	Hernàndez-Ledesma and others 2002
Unnamed	Goat β-lactoglobulin f(103–105)	LLF	Thermolysin	Hernàndez-Ledesma and others 2002

[1]The one-letter amino acids codes were used; Phosphoserine = S*.
[2]Unless otherwise indicated, the precursor protein is from bovine milk.

others 1994a) and in casein hydrolysates produced by *Lb. helveticus* CP790 extracellular proteinase (Yamamoto and others 1994b). In a placebo-controlled study, the blood pressure of hypertensive patients decreased significantly after 4 and 8 weeks of daily ingestion of 95 mL of sour milk that contained the two tripeptides; the ingested dose of ACE-inhibitory peptides ranged from 1.2 to 1.6 mg/day (Hata and others 1996). The effect of two fermented milks started by two *Lb. helveticus* strains also showed an *in vivo* ACE inhibition on normotensive rats (Fuglsang and others 2003). The pressor effect of angiotensin I (0.3 μg/kg) upon intravenous injection was significantly lower when rats were pre-fed with fermented milks. An increased response to bradykinin (10 μg/kg, intravenously injected) was observed using one of the two fermented milks. Also from this study, it was concluded that *Lb. helveticus* produces substances, which *in vivo*, can give rise to an inhibition of ACE. Sodium caseinates prepared from cow, sheep, goat, pig, buffalo, or human milk were hydrolyzed by a partially purified proteinase of *Lb. helveticus* PR4 (Minervini and others 2003). Various ACE-inhibitory peptides were found in the hydrolysates: the bovine α_{s1}-CN f24–47, α_{s1}-CN f169–193, and β-CN f58–76; ovine α_{s1}-CN f1–6, α_{s2}-CN f182–185, and α_{s2}-CN f186–188; caprine β-CN f58–65 and α_{s2}-CN f182–187; buffalo β-CN f58–66; and a mixture of three tripeptides originating from human β-CN. The highest ACE-inhibitory activity of some peptides corresponded to the concentration of the ACE inhibitor (S)-*N*-(1-[ethoxycarbonyl]-3-phenylprolyl)-ala-pro maleate (enalapril) of 49.253 μg/mL (100 μmol/L). UHT milk fermented with the probiotic *Lactobacillus* GG strain and subsequently digested by pepsin and trypsin enzymes has been found to contain ACE-inhibitory activities (Rokka and others 1997). Selected *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactococcus lactis* subsp. *cremoris* were used to produce two fermented milks that contained ACE-inhibitory peptides (Gobbetti and others 2000). The ACE-inhibitory activity of the fermented milk started by *Lb. delbrueckii* subsp. *bulgaricus* SS1 involved peptides such as β-CN f6–14, β-CN f7–14, β-CN f73–82, β-CN f74–82, and β-CN f75–82, The activity in the fermented milk produced by *Lc. lactis* subsp. *cremoris* FT4 had β-CN f7–14 in common but differed with fragments such as β-CN f47–52, β-CN f169–175, κ-CN f152–160, and κ-CN f155–160. All the above CN fragments had a higher proportion of hydrophobic residues (>60%). In particular, β-CN f73–82 and related intermediates contained the internal sequences (Val-Pro-Pro or Ile-Pro-Pro), previously isolated from the Calpis sour milk.

Meisel and others (1997) reported the presence of ACE-inhibitory peptides of low molecular mass in several ripened cheeses. In this study it was suggested that the ACE-inhibitory activity increases as proteolysis develops, but the ACE-inhibition index decreases when the proteolysis during cheese maturation exceeds a certain level (e.g., the ACE-inhibitory activity detected in medium-aged Gouda was about double that of the long-ripened Gouda cheese). Cheeses with low to medium time of ripening contained ACE-inhibitors and the peptides identified in Crescenza cheese corresponded to α_{s1}-CN f23–34 and α_{s1}-CN f23–27 (Maruyama and Suzuki 1993; Maruyama and others 1985, 1987a). The above findings agreed with a previous study (Smacchi and Gobbetti 1998) which demonstrated the presence of ACE-inhibitory peptides in Crescenza, Italico, and Gorgonzola cheeses. β-CN f58–72, which includes the sequence of β-casomorphin-7, was detected by immunoblotting and inhibitory ELISA in Cheddar, Jarlsberg, Norvegia, and Blue cheeses (Stepaniak and others 2001). ACE inhibitory peptides were also found in enzyme-modified cheese (EMC) obtained by hydrolysis of pasteurized cheese homogenized with Neutrase and enzyme preparation from *Lactobacillus casei* (Haileselassie and others 1999). A new type of ripened, low-fat cheese with bioactive properties

was manufactured by using *Lactobacillus acidophilus* and bifidobacteria as starter cultures (Ryhänen and others 2001). The ripened cheese was found to contain peptides with potential antihypertensive effects. Different types of Manchego cheese, manufactured by using different bacterial starters, showed *in vitro* ACE-inhibitory activity (Gòmez-Ruiz and others 2002). The highest ACE inhibition was found for the cheeses manufactured from raw milk and from pasteurized milk inoculated with *Lc. lactis* subsp. *lactis* and *Leuconostoc mesenteroides* subsp. *dextranicum*. Several peptides were identified as fragments of α_{s1}-, α_{s2}-, and β-CN which showed a large number of hydrophobic residues at the C-terminal positions. The peptide corresponding to β-CN f199–204 was chemically synthesized. It showed an *in vitro* ACE-inhibitory activity with IC_{50} of 397.4 μg/mL. The ACE-inhibitory and antihypertensive activities of water-soluble peptides from six representative bacterial (Gouda, Emmental, Edam, and Havarti) and moldy (Blue and Camembert) ripened cheeses were evaluated by *in vitro* and *in vivo* experiments (Saito and others 2000). Although a statistically significant decrease in the systolic blood pressure of spontaneously hypertensive rats was reported for Edam also, Havarti and Blue cheeses had the strongest depressive effect on the systolic blood pressure. Contrarily to what was reported by Meisel and others (1997), the highest ACE inhibitory activity was found for long ripened (8 months) Gouda cheese. Several peptides were identified from cheeses and chemically synthesized. Two peptides, α_{s1}-CN f1–9 and β-CN f60–68, showed a potent ACE inhibitory activity with IC_{50} of 13.4 and 14.8 μmol/L, respectively.

Ovine and caprine β-lactoglobulin were hydrolyzed with trypsin, chymotrypsin, proteinase K, and thermolysin. Four novel ACE-inhibitory peptides were purified and identified from caprine β-lactoglobulin digested with thermolysin: f46–53, f58–61, f103–105, and f122–125. The IC_{50} ranged from 34.7 to 2470 μmol/L (Hernàndez-Ledesma and others 2002). A tryptic digest of bovine α_{s2}-CN contained several casokinins. Peptides corresponding to α_{s2}-CN f174–181 and f174–179 had IC_{50} values of 4 μmol/L. Surprisingly, deletion of the C-terminal dipeptide of the above casokinins did not significantly alter their inhibitory activity (Tauzin and others 2002). A tryptic hydrolysate of CN generated vasoactive or vasopotentiating peptides, γ-CN f108–113 and f114–121, which displayed a selective potentiating activity on isolated guinea pig ileum for bradykinin (Perpetuo and others 2003). Besides, the γ-CN f114–121 showed an *in vitro* competitive inhibition of ACE and thimet oligopeptidase, and presented opiate-like activity.

The majority of CN-derived ACE-inhibitory peptides do not have ACE inhibitory potencies approaching that of captopril (IC_{50} of 0.006 μmol/L) but are characterized by moderate values of IC_{50} in the range of 100–500 μmol/L. However, being naturally derived, BPs would be expected not to have the side-effects associated with synthetically produced drugs used in the control of hypertension, for example, cough and alterations in serum lipid metabolism (Seseko and Kaneko 1985; Nakamura 1987). They also may be of nutritive/physiological importance in that ACE-inhibitory peptides could be active following oral administration (FitzGerald and Meisel 2000). It is noteworthy that Yamamoto and others (1999) demonstrated that Tyr-Pro, having an IC_{50} of 720 μmol/L, could mediate a significant hypotensive effect in spontaneously hypertensive rats. It should be considered that relatively high amounts (10–60 mg) of bioactive peptides could potentially be produced during proteolysis of 1 g of each of the major CN and whey protein components (Meisel 1998). Nevertheless, very highly active BPs were also found. They corresponded to casokinins such as α_{s1}-CN f23–27 and f1–9, β-CN f60–68 and f177–183, and α_{s2}-CN f174–181 and f174–179 having IC_{50} values lower than 20 μmol/L (Saito and others 2000; Meisel 2001; Tauzin and others 2002).

The antihypertensive potential of BPs is dependent on the ability of these fragments to reach their target site without being degraded and as a consequence inactivated by the action of intestinal or plasma peptidases. Inhibition of ACE in lung, vascular, kidney, and brain tissue by captopril, a drug commonly used in the control of blood pressure, is thought to be central to the antihypertensive effect (Unger and others 1985). Resistance to peptidase degradation is a prerequisite for an antihypertensive effect during the oral ingestion and intravenous infusion of ACE inhibitory hydrolysates/peptides. For instance, α_{s1}-CN f23–27, a potent ACE inhibitor *in vitro*, was shown to have no hypotensive effect *in vivo* (Maruyama and others 1987). The presence of Val-Pro-Pro and Ile-Pro-Pro in heat-treated solubilised aortal fractions of spontaneously hypertensive rat fed with Calpis sour milk demonstrated the resistance of these peptides to intestinal and circulatory peptidases, in addition to absorption of these peptides from the intestine (Masuda and others 1996). Proline-containing peptides are generally resistant to degradation by digestive enzymes (Adibi and Kim 1981).

70.9 PEPTIDES INHIBITORY TO BACTERIAL PROTEOLYTIC ENZYMES

Since the antihypertensive activity attributed to BPs has a mechanism based on the inhibition of target proteolytic enzymes, and since proteolytic enzymes from lactic acid bacteria produce BPs in several dairy products, it may be not surprising if a similar mechanism may influence the microbial and enzyme dynamics in food ecosystems. Indeed, once liberated, BPs or peptides related to bioactive sequences may influence the biochemical activity of the microbial communities. Although, in the absence of a clear *in vivo* evidence of the link between peptides inhibiting microbial enzymes and bioactivity, this fact has probably been underestimated in dairy processing (Gobbetti and others 2002). As an example, β-CN f58–72, containing the sequence of β-casomorphin-7, is released during Cheddar, Jarlsberg, and Crescenza ripening and inhibits noncaseolytic oligopeptidase PepO and aminopeptidase PepN of lactic acid bacteria (Stepaniak and others 1995; Smacchi and Gobbetti 1998). β-CN f193–209 and β-CN f194–209 produced by proteinase of *Lc. lactis* subsp. *cremoris* possess antihypertensive effect and inhibit endopeptidases and aminopeptidases of other lactic acid bacteria. Peptidases of lactic acid bacteria differ in their sensitivity to the same peptides (Gobbetti and others 2002, 2004).

70.10 ANTIMICROBIAL PEPTIDES

It is generally accepted that the total antibacterial effect in milk is greater than the sum of the individual contributions of immunoglobulin and nonimmunoglobulin (lactoferrin lactoperoxidase, and lysozime) defence proteins or peptides. This may be due to the synergistic activity of naturally occurring proteins and peptides, in addition to peptides generated from inactive protein precursors (Clare and Swaisgood 2000). Antimicrobial peptides are observed throughout nature. In mammals, they are found both at the epithelial surfaces and within granules phagocytic cells. They are an important component of innate defences, since in addition to killing microorganisms, they are able to modulate inflammatory responses (Devine and Hancock 2002). With the growing problem of pathogenic

organisms that are resistant to conventional antibiotics, there is an increased interest in the application of antimicrobial peptides in food preservation and in reducing the risk of infection.

During the last decades a number of antibacterial peptides encoded in the primary structure of milk proteins have been described. Antibacterial properties, origin and isolation procedure of such peptides are shown in Table 70.3. Lactenin was probably the first antibacterial factor derived from milk that had been treated with rennet (Jones and Simms 1930). The nature or the origin of this inhibitor of streptococcal growth was not elucidated, the antibacterial activity was found to remain together with the whey protein fraction after rennet treatment, and the active component appeared to be resistant to tryptic hydrolysis. A group of basic, glycosylated, and high-molecular-weight (approximately 5 kDa) polypeptides, called casecidins, are released from chymosin-treated CN (Lahov and others 1971). These peptides were shown to display bactericidal properties against lactobacilli and also against various pathogenic bacteria such as *Staphylococcus aureus*. Another antibacterial peptide derived from α_{s1}-CN treated with chymosin, called isracidin, which corresponded to the N-terminal fragment of this protein (f1–23), was isolated (Hill and others 1974). Isracidin was found to inhibit the *in vitro* growth of lactobacilli and other Gram-positive bacteria, but only at relatively high concentrations (0.1–1 mg/mL). However, *in vivo*, isracidin exerted a strong protective effect against *S. aureus*, *Streptococcus pyogenes*, and *Listeria monocytogenes* when administered at doses as low as 10 μg per mouse prior to bacterial challenge. This peptide also safeguarded sheep and cows against mastitis when injected into the udder at levels comparable to those observed with standard antibiotic treatment. Bovine α_{s2}-CN was also shown to be a precursor of several peptide fragments with antibacterial activity. A positively charged peptide, corresponding to α_{s2}-CN f150–188, which was isolated from boiled and acidified milk and called casocidin-I (Zucht and others 1995), showed inhibitory activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus carnosus*) bacteria. Subsequently, the antibacterial role of α_{s2}-CN was reevaluated with the objective of verifying the existence of an enzymatically induced antibacterial domain (Recio and Visser 1999). Hydrolysis of α_{s2}-CN with the gastric enzyme pepsin rendered two different antibacterial peptides: one, f164–179, which was included in the casocidin-I, and a new antibacterial fragment at the C-terminus of the protein, f183–207. The latter had a consistently higher activity than f164–179, although both peptides showed a comparable hemolytic effect. It has also been proposed that part of the antibacterial activity of human milk resides in the CN fraction, particularly in glycosylated κ-CN and its glycosylated region, the caseinomacropeptide (Stromqvist and others 1995). Two reports (Liepke and others 2001; Malkoski and others 2001) have shown a direct bacterial growth-inhibition effect of κ-CN-derived peptides, this activity being unrelated to the presence of attached sugar residues in their amino acid sequences. One of these peptides, called kappacin, corresponded to nonglycosylated, phosphorylated bovine caseinomacropeptide (κ-CN f106–169), which exhibited growth-inhibitory activity against Gram-positive (*Streptococcus mutans*) and Gram-negative (*Porphyromonas gingivalis*) bacteria. It also showed the capacity to bind enterotoxins and to inhibit viral and bacterial adhesion, for example, the binding of cariogenic bacteria to oral surfaces. Lactoferricin, an antibacterial peptide isolated from a peptic hydrolysate of bovine and human lactoferrin (f17–41 and f1–47, respectively), has shown inhibition against a wide variety of microbial species, including many important foodborne pathogens such as *L. monocytogenes* (Bellamy and others 1992; Malkoski and others 2001). Lactoferricin demonstrated higher bacteriostatic

TABLE 70.3 Antimicrobial Peptides Derived from Milk Proteins.

Name	Fragment[2]	Sequence[1]	Antimicrobial Activity	Enzyme or Origin	References
Lactenin	Unknown	Unknown	Pathogenic Streptococci	Bovine milk, rennet	Jones and Simms 1930
Casecidins	Several fragment from κ- and α_{S1}-CN	Unknown	Several pathogenic Strains of *Staphylococcus aureus* and lactobacilli	Chymosin or heat treatment	Lahov and others 1971; Lahov and Regelson 1996
Isracidin	α_{S1}-CN f(1−23)	RPKHPIKHQGLP QEVLNENLLRF	Several microorganisms	Chymosin	Hill and others 1974; Lahov and Regelson 1996
Unnamed	Cow and sheep α_{S1}-CN f24−33	FVAPFPEVFG	Several Gram-positive bacteria *Escherichia coli*	Canestrato Pugliese and Crescenza cheese	Rizzello and others 2004
Unnamed	Sheep α_{S1}-CN f(10−21)	GLSPEVLNENLL	Several Gram-positive bacteria *Escherichia coli*	Pecorino Romano cheese	Rizzello and others 2004
Unnamed	Sheep α_{S1}-CN f22−30	RFVVAPFPE	Several Gram-positive bacteria *Escherichia coli*	Pecorino Romano cheese	Rizzello and others 2004
Unnamed	Goat α_{S1}-CN f24−30	VVAPFPE	Several Gram-positive bacteria *Escherichia coli*	Caprino del Piemonte cheese	Rizzello and others 2004
Casocidin-I	α_{S2}-CN f(150−188)	KTKLTEEEKNRLNFLKK ISQRYQKFALPQYLKTV YQHQK	*Staphylococcus carnosus* *Escherichia coli*	Boiling and acidification	Zucht and others 1995
Unnamed	α_{S2}-CN f(164−179)	LKKISQRYQ KFALPQYLKT	Several Gram-positive and Gram-negative bacteria	Pepsin	Recio and Visser 1999
Unnamed	α_{S2}-CN f(183−207)	VYQHQKAMKPWIQPKTK VIPYVRYL	Several Gram-positive and Gram-negative bacteria	Pepsin	Recio and Visser 1999

(*Continued*)

TABLE 70.3 *Continued.*

Name	Fragment[2]	Sequence[1]	Antimicrobial Activity	Enzyme or Origin	References
Unnamed	Human β-CN f(184–210)	QELLLNPTHQYPVTQPLAPVH NSIV	Several Gram-positive bacteria *Escherichia coli*	*Lactobacillus helveticus* PR4 proteinase	Minervini and others 2003
Unnamed	Goat β-CN f183–187	MPIQA	Several Gram-positive bacteria *Escherichia coli*	Caprino del Piemonte cheese	Rizzello and others 2004
Kappacin	κ-CN f(106–169)	MAIPPKKNQD KTEIPTINTIAS* GEPTSTPTTEA VESTVATLEDS *PEVIESPPEI NTVQVTSTAV[5](non glycosylated, mono- or bi-phosphorylated)	*Streptococcus mutans* *Porphyromonas gingivalis* *Escherichia coli*	Chymosin	Malkoski and others 2001
Unnamed	Human κ-CN f(43–97)	YQRRPAIANNPYVPRTYYAN PAVVRPHAQIPQR- QYLPNSHP PTVVRRPNLHPSF	*Staphylococcus carnosus* *Escherichia coli*	Pepsin	Liepke and others 2001
Unnamed	Cow and sheep κ-CN f(106–115)	MAIPPKKNQD	Several Gram-positive bacteria *Escherichia coli*	Canestrato Pugliese and Crescenza cheeses	Rizzello and others 2004
Unnamed	α-Lactalbumin f(1–5)	EQLTK	Several Gram-positive bacteria	Trypsin	Pellegrini and others 1999
Unnamed	α-Lactalbumin f(17–31)S-S(109–114)	GYGGVLSPEWVC[a]TTF ALC[a]SEK[6]	Several Gram-positive bacteria	Trypsin	Pellegrini and others 1999
Unnamed	α-Lactalbumin f(61–68)S-S(75–80)	C[a]KDDQNPH ISC[a]DKF[6]	Several Gram-positive bacteria	Chymotrypsin	Pellegrini and others 1999
Unnamed	β-Lactoglobulin f(15–20)	VAGTWY	Several Gram-positive bacteria	Trypsin	Pellegrini and others 2001
Unnamed	β-Lactoglobulin f(25–40)	AASDISLLDAQSAPLR	Several Gram-positive bacteria	Trypsin	Pellegrini and others 2001

Unnamed	β-Lactoglobulin f(78–83)	IPAVFK	Several Gram-positive bacteria	Trypsin	Pellegrini and others 2001
	β-Lactoglobulin f(92–100)	VLVLDTDYK	Several Gram-positive bacteria	Trypsin	Pellegrini and others 2001
Lactoferricin-B	Lactoferrin f(17–41/42)	FKC[a]RRWQWRMKKLGAPSI TC[a]VRRAF/A[6]	Several Gram-positive and Gram-negative bacteria	Pepsin or chymosin	Bellamy and others 1992; Jones and others 1994; Dionysius and Milne 1997; Recio and Visser 1999; Hoek and others 1997
Unnamed	Lactoferrin f(1–16)S-S(43–48)	APRKNVRWC[a]TISQPEW LEC[a]IRA[6]	Several Gram-positive and Gram-negative bacteria	Pepsin	Dionysius and Milne 1997
Unnamed	Lactoferrin f(1–42)S-S(43–48)	APRKNVRWC[a]TISQPEWFK C[b]RRWQWRMKKL GAPSITC[b] VRRAFALEC[a]IRA[6]	*Escherichia coli*	Pepsin	Dionysius and Milne 1997
Unnamed	Lactoferrin f(1–16)S-S(17–48)	APRKNVRWC[a] TISQPEW FKC[b]RRWQW RMKKLGAPSITC[b] VRRAFALEC[a]IRA[6]	*Escherichia coli*	Chymosin	Hoek and others 1997
Unnamed	Lactoferrin f(1–16)S-S(45–48)	APRKNVRWC[a] TISQPEW C[a]IRA[6]	*Micrococcus flavus*	Pepsin	Recio and Visser 1999
Unnamed	Lactoferrin f(1–11)S-S(17–47)	APRKNVRWC[a]TI FKC[b]RRWQWR MKKLGAPSITC[b] VRRAFALEC[a]IRA[6]	*Micrococcus flavus*	Pepsin	Recio and Visser 1999
Unnamed	Lactoferrin f(17–30)	FKCRRWQWRMKKLG	Several oral pathogenic bacteria	Synthetic	Groenink and others 1999
Unnamed	Lactoferrin f(19–37)	CRRWQWRM KKLGAPSITCV	Several oral pathogenic bacteria	Synthetic	Groenink and others 1999

(*Continued*)

TABLE 70.3 *Continued.*

Name	Fragment[2]	Sequence[1]	Antimicrobial Activity	Enzyme or Origin	References
Lactoferricin-C	Goat lactoferrin f(14–42)	PEWSKC[a]YQWQRRMRKL-GAPS ITC[a]VRRTSA[6]	*Micrococcus flavus* *Escherichia coli*	Pepsin	Recio and Visser 2000
Lactoferricin-C	Goat lactoferrin f(17–41)	SKCYQWQRRMRKLGAPS ITCVRRTS	*Staphylococcus aureus* *Escherichia coli*	Synthetic	Vorland and others 1998
Lactoferricin-M	Murine lactoferrin f(17–41)	EKCLRWQ-NEMRKVGGPPLSCV KKSS	*Staphylococcus aureus* *Escherichia coli*	Synthetic	Vorland and others 1998
Lactoferricin-H	Human lactoferrin f(1–11)S-S(12–47)	GRRRRSVQWC[a]AVSQPEAT KC[b]FQWQRNMRKVR GPPVSC[b] IKRDSPIQC[a]I[6]	Several Gram-positive and Gram-negative bacteria	Pepsin	Bellamy and others 1992
Unnamed	Human lactoferrin f(18–31)	TKCFQWQRNMRKVR	Several Gram-positive and Gram-negative bacteria	Synthetic	Groenink and others 1999
Unnamed	Human lactoferrin f(20–38)	CFQWQRNM RKVRGPPVSCI	*Prevotella intermedia*	Synthetic	Groenink and others 1999
Unnamed	Human lysozyme f(87–115)	DNIADAVACAKRVVRDPQG IRAWVAWRNR	Several Gram-positive and Gram-negative bacteria	Synthetic	Ibrahim and others 2001

[1]The one-letter amino acids codes were used; Phosphoserine = S*.
[2]Unless otherwise indicated, the precursor protein is from bovine milk.
[5]Sequence corresponding to genetic variant A. In variant B, threonine is substituted by isoleucine and aspartic acid by alanine.
[6]Cysteine residues with the same superscript (a or b) are linked by a disulfide bridge.

and bactericidal activity than intact lactoferrin. Proteolytic digestion of bovine β-lactoglobulin by trypsin yielded four peptide fragments with bactericidal activity. These peptides corresponded to β-lactoglobulin f15–20, f25–40, f78–83, and f92–100 (Pellegrini and others 2001). The four peptides were synthesized and found to exert bactericidal effects against Gram-positive bacteria only. In order to understand the structural requirements for antibacterial activity, the amino acid sequence of the f92–100 was modified. The replacement of the Asp residue by Arg and the addition of a Lys residue at the C-terminus enlarged the bactericidal activity to Gram-negative bacteria. This study showed a possible antimicrobial function of β-lactoglobulin after its partial digestion by endopeptidases of the pancreas and showed, moreover, that small targeted modifications in the protein sequence could be useful to increase its antimicrobial function. More recently, antimicrobial peptides were designed by using combinatorial libraries (Blondelle and Lohner 2000) and novel antibacterial peptides were expressed *in vivo* in *E. coli* (Walker and others 2001) or purified from a pepsin digest of human milk which corresponded to κ-CN f63–117 (Liepke and others 2001). An antimicrobial peptide, corresponding to β-CN f184–210, was produced by hydrolysis of the human sodium caseinate with the partially purified proteinase of *Lb. helveticus* PR4 (Minervini and others 2003). Inhibition was found against potentially pathogenic bacteria of clinical interest, such as *Enterococcus faecium*, *Bacillus megaterium*, *E. coli*, *L. innocua*, *Salmonella* spp., *Yersinia enterocolitica*, and *S. aureus*. β-CN f184–210 inhibited the indicator culture, *E. coli* F19, at a minimal inhibitory concentration (MIC) of 50 μg/mL, which compares well with the lethal concentrations, 4–10 μg/mL, of the more potent antimicrobial peptides (Hancock and Leher 1998). Water-soluble extracts of nine Italian cheese varieties were assayed for antimicrobial activity towards Gram-positive and -negative bacterial species, including potentially pathogenic bacteria of clinical interest (Rizzello and others 2005). Extensively proteolyzed Parmigiano Reggiano, Fossa, and Gorgonzola cheeses did not show antibacterial peptides. Pecorino Romano, Canestrato Pugliese, Crescenza, and Caprino del Piemonte contained a mixture of antimicrobial peptides with a rather high degree of homology. Pasta filata cheeses such as Caciocavallo and Mozzarella also contained antimicrobial peptides. Peptides showed high levels of homology with N-, C-terminal or whole fragments of well known antimicrobial and/or multifunctional peptides reported in the literature: α_{S1}-casokinin (e.g., sheep α_{S1}-CN f22–30 of Pecorino Romano and cow α_{S1}-CN f23–32 of Canestrato Pugliese); isracidin (e.g., sheep α_{S1}-CN f10–21 of Pecorino Romano); kappacin and casoplatelin (e.g., cow κ-CN f106–115 of Canestrato Pugliese and Crescenza); and β-casomorphin-11 (e.g., goat β-CN f60–68 of Caprino del Piemonte). Crude fractions, containing antimicrobial peptides, were inhibitory at a concentration of 30–120 μg/mL.

The activity of most antibacterial peptides can be broadly defined as a membrane-lytic activity, where they tend to assemble to form channels, with specificity for prokaryotic cell membranes. Many BPs have α-helical structures, are cationic and amphipathic, but hydrophobic α-helical peptides and β-sheet peptides possess antimicrobial activity also (Epand and Vogel 1999). Similar structural characteristics of isracidin and lactoferricin (Yamauchi and others 1993; Lahov and Regelson 1996) concern: length of the amino acid chain (23–26 amino acids), low positive charge, high content of nonpolar hydrophobic residues, and some Pro residues very near the C-terminal end of the peptide which could act to make its degradation by peptidases more difficult (Walker 2001). Most antimicrobial peptides interact with membranes and may be cytotoxic as a result of disturbance of the bacterial inner and outer membranes. Alternatively, a necessary but not sufficient property of these peptides is the capacity to pass through the membrane to reach a target inside the cell (Floris and others 2003). The positive net charge property is proposed to initiate peptide interaction

with the negatively charged bacterial surface, while a certain hydrophobic character would permit the peptides to enter the membrane interior (Wieprecht and others 1997). It is not likely that the diverse groups of peptides has a single mechanism of action, but interaction with membranes is an important requirement for most, if not all, antimicrobial peptides (Epand and Vogel 1999). Most antimicrobial peptides bind avidly to lipopolysaccharides of Gram-negative bacteria and lipoteichoic acids of Gram-positive, and are able to depolarize and permeabilize membranes (Devine and Hancock 2002). Under these circumstances, it is possible that the peptides induce the complete lysis of the microorganism by rupture of the membrane or that they perturb the membrane lipid layer, which allows the leakage of certain cellular components as well as dissipating the electrical potential of the membrane.

Antimicrobial peptides have unusually broad activity spectra compared to other agents, with very few intrinsically resistant bacteria. Although more potent antibiotics are available, antimicrobial peptides show the advantages of being able to kill target cells rapidly and having a broad spectrum of activity, including activity for some of the most serious antibiotic-resistant pathogens in clinics. Since the rate of killing is higher than the rate of bacterial multiplication, this enhances the potential to overcome drug resistance (Bechinger 1997).

70.11 CONCLUSIONS

The use of milk protein-derived BPs for application within food industry looks promising for consumers and producers. An important task for the production of functional foods containing BPs is to enhance their bioavailability from dairy products or to create novel foods by addition/fortification of isolated or enriched fractions of BPs. The production of BPs during food processing by the use of food-grade (GRAS) microorganisms such as lactic acid bacteria is of interest for current and future research work. ACE-inhibitory peptides, CPPs, and immunomodulatory peptides are the most suitable BPs for application in foodstuffs formulated to provide specific health benefits. Antimicrobial peptides have received little attention, but because of their useful intrinsic characteristics, an increased application is warranted. Under these perspectives, it seems crucial for the immediate exploitation of the: (1) *in vivo* mechanism by which bioactive peptides exert their activity; (2) intrinsic resistance to digestive enzymes; (3) adaptability and/or resistance to novel and traditional food technologies; and (4) emerging biotechnology for enriching the yield in bioactive peptides.

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71

Science and Technology of Sour Cream

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71.1 Summary 519
71.2 Introduction 520
71.2.1 History 520
71.2.2 Chemical and Physical Background 520
71.3 Legal Definitions 521
71.4 Economical Significance 522
71.5 Ingredients and Additives 523
71.6 Cultures 524
71.7 Manufacturing and Packaging 526
71.7.1 Manufacturing 526
71.7.2 Packaging 530
71.8 Usage 531
71.9 Quality, Analysis, and Consumer Safety 532
References 535

71.1 SUMMARY

According to FAO/WHO, fermented cream (sour cream, cultured cream) is the milk product obtained by fermentation of cream, reconstituted cream or recombined cream, by the action of suitable microorganisms that results in reduction of pH with or without coagulation. Where the content of (a) specific microorganism(s) is (are) indicated, directly

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or indirectly, in the labeling or otherwise indicated by content claims in connection with sale, these shall be present, viable, active, and abundant in the product to the date of minimum durability. If the product is heat-treated after fermentation the requirement for viable microorganisms does not apply.

A process line for production of cultured cream manufacturing includes equipment for standardization of the fat content, homogenization, heat treatment of the cream, and also inoculation, fermentation, and packaging.

Standardized cream becomes heated to at least 80–90°C (at least 5 min holding) before deaeration and homogenization at high pressure (>130 bar) and high temperature (60°C) to improve the texture.

After cooling to mesophilic temperatures (18–25°C) inoculation of 1–4% starter culture takes place in a tank. Fermentation may proceed in a tank or, more commonly, in the final retail pack. The consistency of sour cream might be adversely affected by post-fermentation handling such as stirring, pumping, and filling. Also, the viscosity of the fermented cream might be very high, therefore making it difficult to pack. This is particularly the case with high fat products. Fermentation proceeds for 16–20 h until 0.6% of lactic acid in the fat free phase is reached and is stopped by chilling to around 4–6°C, that is, the packs become transferred to the cold store where they are allowed to rest for 24 h before distribution progresses.

For high-fat sour cream products the homogenization pressure shall be lowered to 100–120 bar only, since there is not enough protein (casein) available to form membranes on the enlarged total surface.

The usage of sour cream is mainly refining dishes. Key criteria defining the quality of the end product are sensorial properties, fat content, and absence of contaminating bacteria. Economically sour cream covers a small but growing segment of the overall fermented milks market.

71.2 INTRODUCTION

71.2.1 History

The history of fermented dairy products including sour cream lies in the dark. According to a legend it shall have been shepherds in the middle east who observed that raw milk permitted to stay for several hours changes in the following ways: (1) Separation of cream from the skimmed milk in a layer on top of the product. (2) Spontaneous fermentation takes place. Hence, since the shepherds appreciated the "new" taste, sour milk products were born such as fermented milk, yogurt, buttermilk, and sour cream.

Other legends claim that an angel delivered yogurt in a pot to earth for the first time, or that a nomad used to carry fresh milk in a container made up of tanned goats skin while riding a camel and recognized that although the product clotted, its sensorial properties were nice and tasty (Klupsch 1992).

Whatever story is true, nowadays fermented dairy products are widely appreciated by the consumers because of their high sensorial properties and nutritional value.

71.2.2 Chemical and Physical Background

Milk basically is made up of water, 4.8% lactose, 3.7% fat, 3.4% protein, mainly the caseins and whey proteins, 0.7% minerals, vitamins, and other so called "minor components."

Native caseins are peptide chains of unfolded threadlike structure with the tendency to form micelles. Within the porous complex the monomers are kept together via hydrophobic bonds, electrostatic bonds (the calcium ion forming bridges between two phosphoro-serin and/or dissociated glutamic acids), and hydrogen bridges. The caseins are widely heat-stable, but coagulate at their isoelectric point of pH 4.6 or via enzymatical cleavage (action of rennet) of the κ-casein. The latter renders the protecting water-soluble glycopeptide to be kept in a dissolved state. The remaining hydrophobic para-κ-casein part of the molecule, however, brings about precipitation and formation of a gel.

Whey proteins such as β-lactoglobulin are globular molecules with three-dimensional structure and are widely pH resistant, however, may unfold under heat treatment conditions (90–95°C for several minutes). The latter property is being used during sour dairy products specific heat treatment conditions of the bulk milk prior to incubation to foster a stable gel formation in the finished good.

Milk may be described as a polydispersed system containing water and dissolved substances in "real" solution, whey proteins and micelles of casein-proteins dispersed in a colloidal state, sometimes small air bubbles, and fat mainly occurring in the form of droplets wrapped by a complex cover of membrane proteins and lipoid substances to keep the droplets dispersed.

Specific mass of raw milk that parameter becomes increased by the dissolved substances, but decreased by the lipid phase in average ending up with a range of 1,029–1,034 g/ml (15°C).

Raw or pasteurized milk may serve as the base for further processing to manufacture different dairy products including consumer milk, fermented dairy products, and others.

Fermented dairy products such as yogurt, sour (clotted) cream, and buttermilk have been manufactured by natural fermentation of the substrate or via the addition of so called starter culture.

Knowledge of the basic chemistry and physics of the proteins and lipids of milk is necessary to understand the processing of fermented milks, yogurt, and sour cream. For further details on chemical, physical, biological, sensory, and nutritive characteristics of milk the reader should refer to Belitz and others (2001), Varnam and Sutherland (2001), and Robinson (2002).

71.3 LEGAL DEFINITIONS

Recommended legal definitions for sweet and sour cream, and cream derived products have been laid down by the FAO/WHO (UN Food and Agricultural Organization/UN World Health Organization) Joint Committee issuing the International Codex Alimentarius (Recommended International Standards for Foodstuffs).

Cream is the fluid milk product comparatively rich in fat, in the form of an emulsion fat-in-skimmed milk (o/w type), obtained by physical separation from milk. The FAO/WHO standard for the fat content of cream is exhibited in Table 71.1.

Fermented cream (sour cream, cultured cream) is the milk product obtained by fermentation of cream, reconstituted cream or recombined cream, by the action of suitable microorganisms that results in reduction of pH with or without coagulation.

Acidified cream is the milk product obtained by acidifying cream, reconstituted cream and/or recombined cream by the action of acids and/or acidity regulators to achieve a reduction of pH with or without coagulation (CODEX STAN A-9-1-Rev. 1-2003).

TABLE 71.1 FAO/WHO Standards for the Fat Content of Cream.

Type of Cream	Min. Fat Content (%)
(Single) cream	18
Half cream	10–18
Double cream	45
Whipping cream	28
Heavy whipping cream	35

More specifically the legal definition for fermented cream in Germany is given as follows:

Cultured cream is the product with a fat content of minimum 10% produced from sweet cream by culturing with lactic acid bacteria. The cream becomes standardized, cooled, and incubated usually with 2–4% butter culture, sometimes also including *Lactobacillus acidophilus*. The cream is fermented at a temperature of 20–27°C until pH of 4.5 is obtained (Pabst 2004).

Labelling criteria are laid down by the Codex General Standard of the Labelling of Prepacked Foods (CODEX STAN 1-1985, Rev. 1-1991; Codex Alimentarius, Volume 1A) and the General Standard for the Use of Dairy Terms (CODEX STAN 206-1999).

In case that nutrition claims are used, labelling needs to be in accordance with the Codex Guidelines for Use of Nutrition Claims (CAC/GL 23-1997, Codex Alimentarius, Volume 1A).

Legal issues concerning ingredients and additives are discussed in different sections below.

71.4 ECONOMICAL SIGNIFICANCE

Throughout the years after World War II until now, the market for fermented dairy products has expanded tremendously. The annual consumption of yogurt per head in Germany used to be 0.95 kg in 1961, however, increased to 12.0 kg in 1991 (Klupsch 1992). As to the cream market, cultured cream covers a small but growing market segment. Table 71.2 provides an idea of the cream market in the United Kingdom

TABLE 71.2 Cream Household Sales and Percentage Change Year on Year (UK 1988).

Name	Volume in 1000 L	% Change Year on Year
Total cream market	40,461	+4.0
Total dairy cream market	36,538	+5.0
Total fresh cream market	24,333	+8.0
Single	6,800	+5.0
Double	10,337	+5.0
Whipping	4,134	+24.0
Clotted	1,541	+13.0
Soured	519	+15.0
Total UHT cream market	6,722	+6.0
Total sterilized cream market	3,676	+1.0
Total frozen cream market	1,807	−12.0
Total synthetic cream market	3,923	−7.0

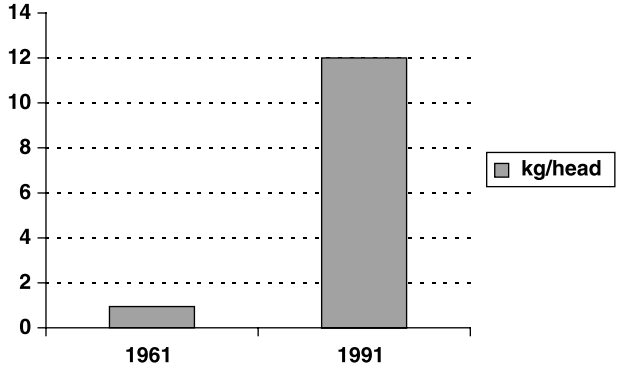

Figure 71.1 *Progress of annual yogurt consumption per head (Germany 1961 versus 1991).*

(Rothwell 1989). Figure 71.1 shows the progress of annual yogurt consumption per head (Germany 1961 vs. 1991).

71.5 INGREDIENTS AND ADDITIVES

Milk and dairy products generally are regarded to serve as basic nutritives in man's diet, therefore, addition of additives is limited to specified technological needs. As to sweet and sour cream FAO/WHO Codex Alimentarius states raw materials as follows (CODEX STAN A-9-1-Rev. 1-2003).

All kinds of cream and prepared cream utilize milk as the main ingredient. Milk for that purpose may have been subjected to mechanical and physical treatments prior to cream processing. According to the Codex the term "cream" is only suitable for products with a minimal butterfat content of 10%.

Only those ingredients listed in the Codex are permitted to be used in the processing of cream, those which are not there are forbidden.

For sour cream only suitable starter cultures are permitted, whereas rennet or equivalent coagulating enzymes and sodium chloride are permitted for fermented and acidified cream.

The additives classes allowed are stabilizers, thickeners, emulsifiers, and acidity regulators if they are needed to ensure the stability and integrity of the emulsion.

Stabilizers and thickeners limited by GMP are alginates, agar-agar, carrageenans, various gums (carob bean, guar, gum arabic, xanthan, gellan), pectin, celluloses, derivatives of celluloses, various native and modified starches. Also functional milk proteins, various milk powders and gelatin are permitted.

Stabilization of cultured dairy products is necessary if natural systems fail to keep the agglomerated casein-micelles (curd) in a three-dimensional network. This is particularly the case for products which become pasteurized (thermization) after filling when undesired flocculation may occur causing sandy mouth feel.

The gel shows a tendency of shrinking which may initiate separation of the whey. This phenomenon is generally called syneresis. Syneresis is often a problem for long shelf-life products.

Factors causing sandiness are as follows:

- No or low denaturation of the whey proteins
- Low fat content

- High thermization temperatures
- Long thermization time.

A natural way to stabilize the system is to increase the product's dry matter by addition of skimmed milk powder before fermentation.

Stabilization by stabilizers is brought about by addition of protective colloids (hydrocolloids). Also gel forming substances, thickeners to increase viscosity, and emulsifiers are being used.

The most common substances which become applied are functional milk proteins (emulsifying properties), native, and modified starches (gel forming and/or thickening properties), and gelatine (gel forming properties). Also carob bean and guar kernel flour (thickening properties) are being used. Sometimes celluloses or carboxy-methyl-celluloses (CMC) are added. Pectin, which is available in a low and in a high-esterified variety, is another hydrocolloid exerting protective-colloid and gel forming properties at low pH values.

Because of the BSE-crisis gelatine has got a poor reputation for European consumers. Thus many dairies replace gelatine by alternative substances as listed above. A combination of pectin and starch, or functional milk proteins probably is most commonly used.

Because of its high fat content sour cream is more stable than yogurt. It is, however, usually desirable to stabilize sour cream too. The substances applied vary with the fat content and the desired viscosity of the product (Fox, Neumann and Popper 2002).

71.6 CULTURES

Lactic acid bacteria (LAB) are Gram-positive facultative anaerobic bacteria fermenting lactose to lactic acid as the main metabolic product. They may be classified according to morphologic criteria (streptococci/lactococci or rods-lactobacillaceae) or according to physiological criteria (homofermentative or heterofermentative). Homofermentative means lactic acid as the more or less single metabolic product (apart from carbondioxide), whereas heterofermentative means that lactic acid is accompanied by other metabolic products such as ethanol or various organic acids.

Taxonomy and physiology of LABs have been reviewed in detail elsewhere (Varnam and Sutherland 2001; Bylund 2003).

In unpasteurized milk, spontaneous fermentation takes place caused by microorganisms (LABs) which are viable within an acid environment, at deep redox-potential, and finally, can utilize lactose as a carbon-source (Klosterneyer 1996).

Commercial sour-milk products use processed milk as the substrate. Acidification is brought about until or below pH values of the isoelectric point of the caseins, coagulation takes place to form the curd.

Nowadays in industrial dairy processing, natural fermentation has been replaced by commercially available blends of specific organisms, which are commonly referred to as "starter cultures." These selected kinds of organisms are responsible to initiate fermentation in the desired way to reach pH of 4.5–4.8 and to generate flavoring substances characterizing the product under concern (Klupsch 1992).

Some strains such as *Bifidobacterium* ssp. are claimed to exert therapeutic, probiotic properties to human health such as maintenance of a normal intestinal microflora,

stimulation of the host's immune system, alleviation of lactose maldigestion, anticarcinogenic activity, or reduction of serum cholesterol levels (Varnam and Sutherland 2001).

A conditio sine qua non for bringing about proper fermentation is that the substrate is free of inhibitory substances, mainly antibiotics originating from veterinary mastitis treatment without complying with minimal waiting periods after the treatment. Thus the processed milk has to be screened for antibiotics prior to fermentation (Brilliant-Black-Reduction test, Delvo test, Charm test or others). Also the milk needs to be free of bacteriophages that might attack the LABs and causes another reason for failure of the starter culture.

The fermentation process is undertaken at mesophilic (sour milk, sour cream) or thermophilic temperatures (yogurt) and, afterwards, stored at cool temperature of 3–5°C, so that it comes to a standstill.

Throughout the last two decades commercially available starter cultures have replaced that ones produced by the creamery itself. Frozen and freeze-dried units have superseeded the liquid cultures. Direct-to-vat inoculation has become the most common feature.

Typical starter cultures contain lactic-acid-bacteria as follows:

1. Sour milk: Mesophilic culture made-up of *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris*. Sometimes *L. lactis* ssp. *lactis* biovar *diacetyllactis* or *Lb. acidophilus* are around too.
2. Yogurt: Thermophilic culture made-up of *Sc. salivarius* ssp. *thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*.
3. Sour cream: "Butter culture" which is a mesophilic culture similar to that for sour milk.

Frozen DVS (direct to vat set) look like pellets; freeze-dried DVS show a granular shape with a diameter of 2–5 mm. The purity of these cultures as guaranteed by the suppliers (Chr. Hansen 2003; Danisco 2003) is given in Table 71.3.

A "classical" mesophilic mixed-strains culture for fermented milk, sour cream and sour cream butter is specified in Table 71.4 (Chr. Hansen 2003).

Progress of acidification needs to be monitored via pH measurement or titration and becomes plotted against time to indicate potential starter failure and to ensure the desired properties of the product (Figure 71.2).

TABLE 71.3 Microbiological Specification of a Common DVS Culture.

Bacteria sp.	Frozen DVS	Freeze-Dried DVS
Coliform bacteria	<1 MPN/g	<10 MPN/g
Enterococci	<10 CFU/g	<100 CFU/g
Molds	<1 CFU/g	<10 CFU/g
Non-LABs	<500 CFU/g	<500 CFU/g
Yeasts	<1 CFU/g	<10 CFU/g
Staphylococcus aureus	<1 CFU/g	<10 CFU/g
Salmonella sp.	Absent in 25 g	Absent in 25 g
Listeria	Absent in 1 g	Absent in 1 g

Source: Chr. Hansen (2004).

TABLE 71.4 Product Description of a Classical Mesophilic Starter Culture.

Product Description	Concentrated Deep-Frozen Starter Culture Pellets for Direct Inoculation into the Process Milk
Type	*Classical mesophilic mixed strains culture*
Composition	*Lactococcus lactis* subsp. *Lactis* *Lactococcus lactis* subsp. *Cremoris Lactococcus lactis* subsp. *lactis biovar diacetyllactis Leuconostoc mesenteroides* subsp. *cremoris*
Properties	Moderate acidification, formation of mainly L(+) lactic acid, slow citrate fermentation, and thus little carbon dioxide release, moderate diacetyl formation (typical flavor)
Packaging	Polyethylene bags
Shelf-life	12 month at $<45^{\circ}C$
Usage	Open the bag right before inoculation, add the culture aseptically and in a frozen state directly into the procesed milk
Application	Sour cream 1.0–2.0 units/100 l, also applicable for butter- and thick-milk-fermentation and some cheeses as well

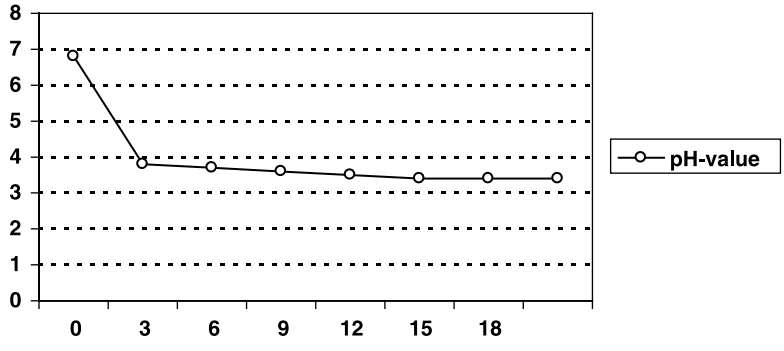

Figure 71.2 *Plotting of pH vs. fermentation time.*

71.7 MANUFACTURING AND PACKAGING

71.7.1 Manufacturing

The basic technology of cream includes separation and standardization, homogenization, pasteurization, and other postseparation processes to obtain creams with differing properties. These basic unit operations have been discussed in relation to cream, its quality, viscosity, stability against syneresis, and other properties by Towler (1987) and Rothwell (1989).

Heat treatment of milk and cream is compulsory to ensure safety of the product, and, in case of sour cream, to bring about denaturation of the whey proteins to achieve a stable curd. Whereas for pasteurized milk applying the HTST (high temperature-short time) process $72^{\circ}C$ for 15 s is regarded to be good enough, because of higher viscosity the heat transfer rate within cream becomes impaired thus more severe heat treatment conditions become arranged such as $85^{\circ}C$ for 30 min or $90-95^{\circ}C$ for 5–10 min to provide two examples.

Usually heat treatment, holding and cooling, but also incubation and addition of fruit preparations become brought about in the same vessel ("multipurpose processing tank") for the stirred type fermented milk, but plate-heat exchangers with an appropriate

holding-tube, incubation in a vessel and fermentation in the final pack is also common for the set type of fermented dairy products.

Commercial sterilization of cream (in the container or UHT-ultra high temperature) requires a reduction rate of 9D (i.e., nine logarithmic cycles) in numbers of endospores-forming bacteria.

Fermented milks may be classified in a number of ways, but a system based on the type of starter microorganism used is generally satisfactory (Table 71.5).

The fat content of cultured (sour) cream is standardized between 10% and 30% depending on the required properties. A process very similar to that used in buttermilk manufacture except that cream of at least coffee cream fat content is used to make sour cream.

Basically sour cream is an extremely viscous product with a gel-like consistency with the flavor and aroma of buttermilk (acetoin as a major flavor component) which is the case because the starter culture is related to that used for cultured buttermilk, that is, mesophilic culture compiled of *L. lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, and *L. lactis biovar diacetyllactis.* This is a balanced mixture of acid and flavor producing bacteria (Desrosier 1970).

The process line for production of cultured cream manufacturing includes equipment for standardization of the fat content, homogenization, heat treatment of the cream, and also inoculation, fermentation, and packaging.

Standardized cream becomes heated to at least 80–90°C (at least 5 min holding) before deaeration and homogenization at high pressure (>130 bar) and high temperature (60°C) to improve the texture.

After cooling to mesophilic temperatures (18–25°C) inoculation of 2–4% starter culture takes place in a tank. Fermentation may be brought about in a tank or, more commonly, in the final retail pack. The consistency of sour cream might be adversely affected by postfermentation handling such as stirring, pumping, and filling. Also, the viscosity of the fermented cream might be very high, therefore making it difficult to pack. This is particularly the case with high-fat products.

Fermentation proceeds for 16–20 h until 0.6% of lactic acid in the fat free phase is reached, and is stopped by chilling to around 4–6°C, that is, the packs become transferred to the cold store where they are allowed to rest for 24 h before distribution takes place (Varnam and Sutherland 2001; Bylund 2003).

TABLE 71.5 Classification of Fermented Milks.

Type of Fermentation	Product
Mesophilic lactic fermentation	Cultured buttermilk
	Cultured cream
	Filmjolk
	Scandinavian ropy milks
Thermophilic lactic fermentation	Yogurt
	Acid buttermilk
Therapeutic lactic fermentation	Acidophilus milk
	Yakult products
	Acidophilus-bifidus yogurts
	Proprietary therapeutic products
Lactic/yeast fermentation	Kefir
	Kumis
Lactic fermentation/mold ripening	Viili

Source: Varnam and Sutherland (2001).

For high-fat sour cream products the homogenization pressure shall be lowered to 100–120 bar only, since there is not enough protein (casein) available to form membranes on the enlarged total surface (Bylund 2003).

When properly refrigerated, sour cream should maintain its smooth consistency for several weeks. Exposure to higher temperatures or holding for more then 4 weeks may cause whey separation. The product is still usable after stirring but at this stage it may be somewhat less appealing in flavor. Addition of hydrocolloids may increase the texture stability as discussed above.

Anyhow, the shelf-life of the cultured cream may be prolonged by heat treatment of the fermented product (long-life cultured cream). Stabilizers need to be added usually into the cream before fermentation to improve resistance of the texture against the final heat treatment. The viscosity of the ready product is dependent on the choice of stabilizer, as well as on the design of the particular plant.

A slightly different but also common schedule for the manufacture of sour cream is given in Figure 71.3 (Robinson 2002).

The optional supplementation with milk or whey powders requires additional deareation and filtering steps. Denaturation of the whey proteins via heating to 90°C with subsequent holding is desired to improve the texture and to avoid postprocessing gelatination in the pack.

Sour cream dip is a cultured cream of 12–16% fat containing dry milk solids, sugar, stabilizer, rennet extract, spices, salt or pepper, and sometimes, various cheese condiments.

Crème fraiche is a French denomination for mildly fermented cream of a fat content of $\geq$30%. This is a variety of cultured cream, which is of premium quality usually taken for refinement of dishes. Crème fraiche is popular in Central Europe complying with quality requirements as follows (Klupsch 1992):

- Natural fermentation
- No addition of stabilizers
- No thermal treatment after fermentation
- Stable against syneresis for 5 weeks shelf-life.

Dairy spreads made from sour cream are cultured cream of minimal 28% fat, which show plastical, butter-like rheological properties. The manufacturing process for pasteurized sour cream spreads is given in Figure 71.4 (Klupsch 1992).

Sour cream powders have a variety of applications such as salad dressings, sauces, dips, frozen vegetables, cake mixes, snack blends, and seasoning bases. Containing 21–54% butterfat the products include full-fat, low-fat, high-acid, and high-flavor versions (Anonymous 1994).

Filled sour cream is a product where butterfat becomes replaced by vegetable fat such as palmolein.

Acidified sour cream is a product using sweet cream as the base, but bringing about low pH values by addition of organic acids instead of a natural acidification process. These products obviously lack the typical flavor produced during the fermentation of lactose if not fixed by addition of specific flavoring components.

Sour cream imitates occasionally may appear in the market (Desrosier 1970). Imitation may refer to the way of achieving acidification and/or the nature of the fat phase. These products, however, are not permitted to be used by the WHO/FAO Codex Alimentarius for potential fraud reasons. Specific labeling is required.

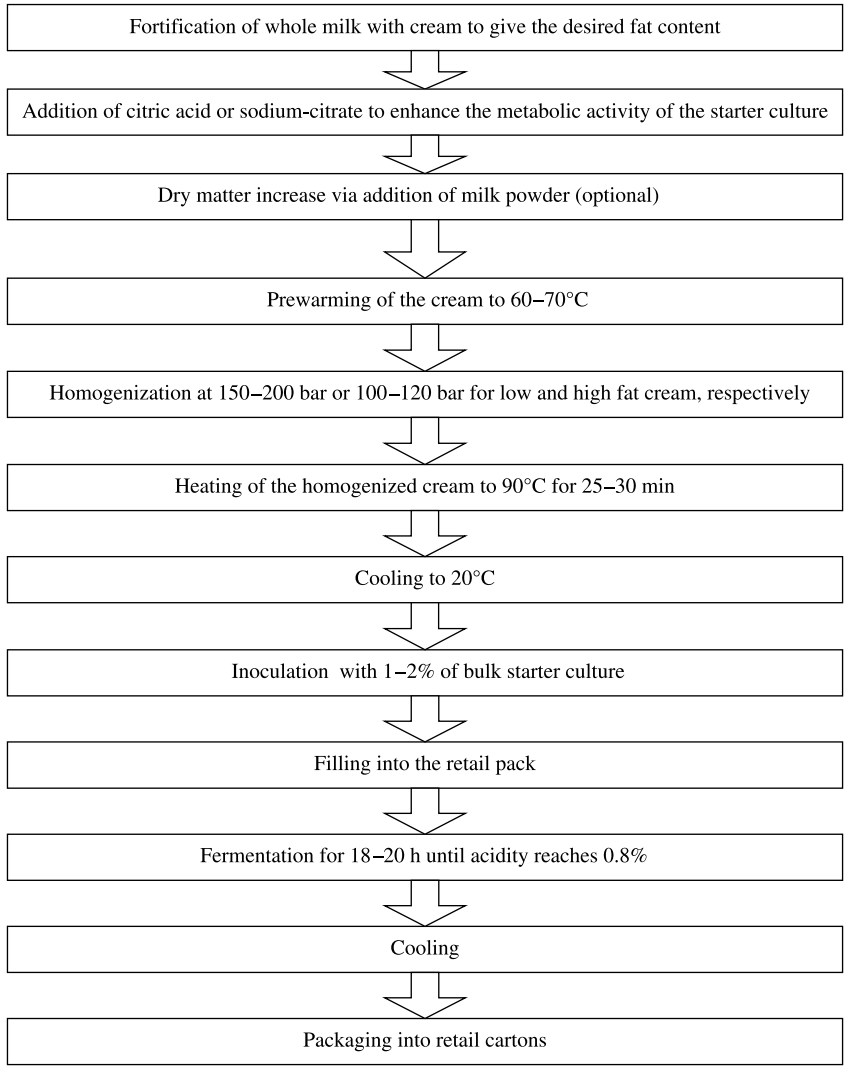

Figure 71.3 *Process flow-chart for sour cream manufacturing acc. to Robinson 2002.*

No fat sour cream is an imitation product where the fat becomes replaced with skimmed milk powder and dairy proteins. The product is claimed to have the full flavor and smooth texture of regular sour cream. This product shall be heat stable within a wide range of time–temperature conditions applied. The unopened shelf life is 4 months in accordance with the information released by the manufacturing company (Anonymous 1995).

Cream yogurts in a way may also be regarded to form a variety of cultured cream. These products are standardized to 10% fat and thermophilic fermentation conditions are applied (40–42°C) using a yogurt starter culture. Fermentation becomes stopped after 4–6 h via cooling to refrigerator temperature. Fermentation might be proceeded in the tank (stirred type) or in the final pack (set type). The process of yogurt making is discussed in more detail by Robinson (2002).

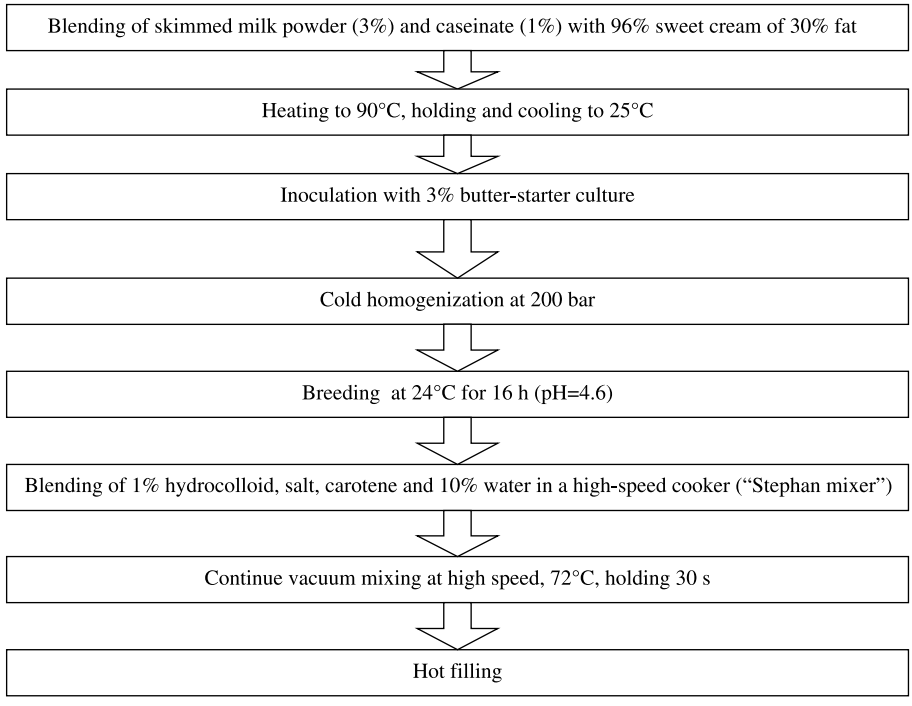

Figure 71.4 *Process flow-chart for pasteurized sour cream spreads.*

Another function of sour cream is to serve as the *raw material of sour cream butter*. Butter is the product gained by dashing concentrated cream to render the fat globules to flow into each other. Thus a phase reversion is achieved from an o/w type emulsion to become a w/o one. Butterfat forms as the continuous phase, small water droplets make up the dispersed particles.

Buttermilk is the liquid low-fat product left over following the separation of the butter grains from the skimmed phase. This by-product of the buttering process is sweet or sour buttermilk, which is usually utilized as feed for cattle and pigs. The commercially available buttermilk, however, is made up by inoculation and fermentation of skimmed milk via usage of a buttermilk starter. The basic types of butter are sweet cream butter and sour cream butter.

Butter made from sour cream has certain advantages over the sweet cream variety. The aroma is richer, the butter yield is higher, and there is less risk of reinfection after temperature treatment as the bacterial culture suppresses undesirable microorganisms. The drawbacks, however, are that it is more sensitive to oxidative fat defects.

Fermentation of sour cream foreseen for the churning process takes place for around 16–20 h at mesophilic temperatures. Sweet buttering cream undergoes a ripening process only to achieve small fat crystals for reducing hardness of the butter.

An excellent review of cream processing and butter making has been provided by Bylund (2003).

71.7.2 Packaging

Material properties requirements for packaging of sour cream are reasonable light and oxygen tightness to avoid light or oxygen induced fat oxidation and thus potential off-flavor, relative impermeability to moisture, proper robustness to avoid mechanical

damage throughout the overall supply chain, tightness to microorganisms and dust particles, and suitability for automatic product filling and packaging at high speed lines. The filled containers must not contaminate the product with flavor-inducing chemical molecules (e.g., styrene monomers). The microbiological contamination of the materials's surface needs to be reasonably low.

Containers to be filled mechanically may usually be polypropylene or polystyrene beakers to be filled hot or cold before fermentation and sealed by aluminum foil. Sometimes glass jars with plastic caps become used. Aseptic filling into polyethylene and metal-layered carton packs is quite common for UHT-cream but less common for sour cream.

Eventually for packaging, the sour cream containers are placed into cardboard cases, which are being moved into the cold store and kept there to rest for 24 h before being ready for distribution. The palettes keeping the cases usually become stabilized by wrapping with polyethylene stretch foil (Rothwell 1989).

71.8 USAGE

Basically there are three main usages of sour cream:

(1) Usage of sour cream in the kitchen to refine various dishes such as addition to sauces and fillings for cakes.
(2) Usage for salad dressings as a comparably low calories replacement of mayonnaise.
(3) Technological usage of high fat cultured cream as the raw material for sour cream butter-making (ref. above).

TABLE 71.6 Sour Cream Salad Dressing.

Ingredients	Quantity
Salt	$\frac{1}{2}$ teaspoon
Sugar	1 teaspoon
Cayenne pepper	$\frac{1}{8}$ teaspoon
Lemon juice	1 tablespoon
Vinegar	2 tablespoons
Sour cream	1 cup

Instruction: Stir together sour cream, vinegar and lemon juice, add sugar and spices, stir again, serve cold.

TABLE 71.7 Recipe Vanilla-Raisin Bread.

Ingredients	Quantity
Golden raisins	1 cup
Vanilla	2 tablespoons
Water	$\frac{1}{2}$ cup
Light sour cream	$\frac{3}{4}$ cup
Honey	3 tablespoons
Salt	1 tablespoon
Bread flour	3½ cups
Cinnamon	1 tablespoon
Active dry yeast	2 tablespoons

Note: Soak the raisins in the vanilla for at least 1 h. Place ingredients 3–9 in a pan, bake on sweet cycle, and add raisins after kneading beep.

TABLE 71.8 Recipe Sour Cream Dip.

Ingredients	Quantity
Shredded hard cheese	2 cups
Sour cream	$\frac{11}{2}$ cups
Sliced black olives	2 small cans
Diced tomatoes	$\frac{11}{2}$ cups

Note: Bake beef or pork meat together with spices almost until done, then add the sour cream in an even layer ontop the meat, then cheese, then olives, bake uncovered until the cheese melts, after taking out of the oven sprinkle tomatoes over the top.

When googling in the internet using "sour cream" as the key words a variety of several hundred URLs appear giving a tremendous amount of recipes of sour cream for cooking purposes. To provide an idea the author selected three examples, which are shown below (Tables 71.6–71.8).

71.9 QUALITY, ANALYSIS, AND CONSUMER SAFETY

For each dairy processing plant, appropriate processes to manage the quality have to be laid down in writing and also to be practically implemented. Such a quality management system may or may not become certified according to the international standard of ISO 9001: 2000 (Peach and others 2000).

Good manufacturing practice (GMP) is a set of basic hygienic rules laid down in SSPs "standard sanitation procedures" or SOPs "standard operational procedures."

A systematic, proactive evaluation of a particular product's process line and its potential usage for consumer safety aspects and afterwards stipulating control points is called Hazard Analyses Critical Control Points (HACCP) (Loken 1995). Critical Control Points to ensure end-product safety (CCPs) for sour cream processing might be: Test for inhibitory substances in the incoming milk, pasteurization, pH values (<4.2) and metal detection.

The analytical chemical, physical, biological, and sensorial methods used in the laboratory or direct at the manufacturing line have to be identified, calibrated properly, and compared with other laboratories via inter-laboratory tests.

Cultured cream is bright, has a uniform structure and is usually viscous. The taste should be mild and slightly acidic with body, creaminess and proper mouth feel. In case of the set type variety of sour cream the texture shall be extraordinary thick and smooth, that is, in a way that a spatula put into the product can stand upright in it.

The rheological features show non-Newtonian flow characteristics, that is, thixotrophic properties. A product is called thixotrophic if exerted shear stress causes decrease of the apparent viscosity (Pa·s) and if the product after being relieved from the shear stress only partly regains its previous structure. This effect observed for viscous-elastic gels is sometimes called shear thinning.

Apparent viscosity may be determined by means of a rotation viscosimeter. The undisturbed gel may become physically investigated for hardness/gumminess, cohesiveness, and adhesiveness to establish a texture profile by using a texture analyzer. Addition of a few ppm of rennet to the sweet cream before adding the starter culture fosters proper gel formation. The gel strength is a maximum at pH values in the area of 4.6, but the

gel strength and firmness decrease at pH < 4.0 because of charge repulsion of the caseins. In case the whey proteins have been denaturated by heat treatment they aggregate with the caseins and hence contribute to a firm gel structure.

Potential reasons for texture problems might be as follows: too high separation temperature ($T > 45^{\circ}C$), too high incubation temperature, insufficient heat treatment so that the whey proteins are not completely denaturated, too low milk solids nonfat (MSNF), too high whey protein to casein ratio, improper selection use and concentration of stabilizers, improper starter cultures (or infection) to cause proteolysis and uncontrolled exopolysaccharide expression of some bacterial strains (Klupsch 1992).

Aspects of syneresis and stabilization of sour cream have been discussed above.

The degree of syneresis may be measured by inverting the product upside down onto a fine sieve and collect and weigh the whey passing through the sieve.

The nutritive value of sour cream of 10% fat is given by 3.1% protein, 10.1% fat, 3.5% carbohydrate (lactose), fat- and water-soluble vitamins, minerals, and trace elements. The energy content of 100 g cultured cream is 510 kJ (122 kCal).

Good raw-milk quality given by low total bacteria count, low somatic cells count, absence of inhibitoring substances, and absence of off-flavor and taste, is essential for the manufacture of a high quality product.

Mechanical damage of the raw or pasteurized milk due to too intensive agitation or pumping needs to be avoided as much as possible, because free fat released from the fat droplets by membrane damage is prone to oxidation. Enzymatic decay may foster a metallic like or rancid off-flavor (Heiler and Schieberle 1996).

Homogenization shall be done at comparably low pressure in two steps to avoid too small droplets corresponding with too large total surface area so that the caseins might not be able to quickly form new intact membranes.

Process control of a sour cream manufacturing line involves fat content measurement of the standardized product, monitoring of the time–temperature conditions applied while heating, monitoring the homogenization pressure applied, testing the activity of the starter culture prior to inoculation, monitoring of fermentation time and temperature, pH or acidity measurement progressing throughout the fermentation, sensorial testing and pH measurement of the fruit preparation, if so applicable.

GMP is confirmed to be attained via sampling for various microbiological tests and implementation of swab tests taken from the milk contacting surfaces or from the environment of the process line.

All operations have to be in accordance with basic hygienic and sanitation rules, particularly postprocessing contamination (PPC) downstream heating needs to be avoided. Any addition of additives or fruit preparation after heating needs also to be monitored. Some authors regard this GMP like process steps to form another CCP (Varnam and Sutherland 2001).

Shelf-life of a sour cream product shall be 4–6 weeks if manufactured in accordance with the hygienic rules described beforehand including constant proper cooling to $4–6^{\circ}C$. After opening the product may remain durable for another 2 weeks. A spoon might remix it in case whey separation occurs (Daisy Dairy Products Ltd. 2004).

An end-product-testing scheme applicable for sour cream and other fermented dairy products widely used in Central Europe has been established by the German Society of Agricultural Sciences (DLG = Deutsche Landwirtschafts-Gesellschaft 2002) as follows.

The fat content of sour cream according to the declaration on the label needs to be at least 10, 20, 30, or 40%. The viscosity of the product in accordance with appropriate

labeling may be liquid, thick, or set. The sensorial properties of the product become evaluated following a 5 points scale for the features appearance, odor, flavor, and texture.

The microbiological quality of the product becomes evaluated with the aid of the parameters coliform germs and molds/yeasts. Coliform bacteria basically need to be less then 10 CFU (colony forming units) in 1 ml samples. Molds and yeasts need to be <100 CFU/mL to achieve the maximal ranking.

The results of sensorial testing and microbiological evaluation become multiplied by a weighing factor, then added up, and finally divided by the overall sum of the weighing factors (i.e., 24 in the DLG scheme) to come up with an overall quality figure, which is in the range of 1–5 points, obviously.

The samples shall have been kept for not more then 2 weeks at 4–8°C. The packs need to be tight. Samples shall have been drawn in accordance with the sampling plan provided by the DLG. The temperature during the conduction of the sensorial tests shall be in the range of 14–18°C. Spoiled samples are to be destroyed.

Analytical methods for examination of dairy products including sour cream have been published by the German Association of Food testing- and Research Institutes (VDLUFA = Verband Deutscher Lebensmittelunter-suchungs- und Forschungsanstalten 1995).

These reference methods are as follows:

(1) Chemical methods
- Fat content: Gravimetric method according to Röse-Gottlieb (Note: In the Anglo-American countries the method usually is called "Mojonnier-method.").
- Protein content: Nitrogen based method according to Kjeldahl.
- Nonprotein-nitrogen: Precipitation of proteins with trichloric-acetic-acid, filtration, nitrogen determination in the filtrate.
- Lactose and galactose content: Enzymatically.
- Mineralic substances: Gravimetric oven-method determining the ash content.
- Sucrose and dextrose content in sweetened dairy products: Enzymatically.
- Number of double bindings in fat: Jodometric titration method ("Jod value"). Routine methods such as IR-spectroscopic determination of fat, protein, and lactose have to be calibrated versus the reference-methods. Nowadays, turbidimetric and IR measurement of fat content have superceded widely the formerly most common butyrometric rapid methods for examination of fat (milcoscan technology).

(2) Microbiological methods
- Total plate count of colony forming units: Koch's petridish method using china blue lactose-agar.
- Coliform germs: MPN (most probable number) method based on sodium laurylsulfate medium or petridish method using violetred-bilesalts-agar.
- Yeasts and molds: Petridish method using maltextract-agar.
- Lipolytic bacteria: Petridish method using tributyrin-agar.

(3) Physical methods
- Apparent viscosity as given above (Adapa and Schmidt 1998).
- Determination of gumminess, adhesiveness and cohesiveness by means of a texture analyzer.

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72

Dairy Protein Hydrolysates

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72.1 Introduction 537
72.2 General Aspects of Protein Hydrolysis 539
72.2.1 Fundamentals of Protein Hydrolysates 539
72.2.2 Analysis of Protein Hydrolysates 540
72.3 Technology of Dairy Protein Hydrolysates 544
72.3.1 Why Hydrolyze Proteins? 544
72.3.2 Types of Dairy Protein Hydrolysates and General Production Process 544
72.3.3 Advances in Processing and Analytical Technologies of New Hydrolysate Products 546
72.4 Functions and Applications of Dairy Protein Hydrolysates 547
72.4.1 Selected Functional Properties of Protein Hydrolysates 548
72.4.2 Application of Dairy Proteins in Selected Nutritional Products 550
72.4.3 Bioactive Peptides from Dairy Proteins and Potential Applications 551
72.5 Future Perspectives 553
References 553

72.1 INTRODUCTION

Protein hydrolysates are produced through hydrolysis (size reduction) of intact proteins to amino acids or mixture of polypeptides, oligopeptides, and smaller peptides and amino acids, depending on the method of hydrolysis. They represent a category of protein

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ingredients with broad applications in food, nutritional, dietary supplement, medical, and personal care products. Protein hydrolysates have been used since the 1940s for the nutritional management of individuals who cannot digest intact protein (Cuthbertson 1950; Mahmoud and others 1992). The most prevalent use of such hydrolysates, however, has been for feeding infants with food hyper sensitivity (particularly cow's milk) (Mahmoud and others 1992). For the past decade, there has been increasing interest in the utilization of native and hydrolyzed milk proteins for human nutrition and health-related foods (De Wit 1998; Floris and others 2003; Kilara and Panyam 2003; Korhonen and Pihlanto 2003; Playne and others 2003).

Hydrolysis of proteins can be accomplished with enzymes, acids, or alkali, but enzymatic hydrolysis is strongly preferred over strictly chemical methods for producing hydrolysates in nutritional applications (Lahl and Braun 1994). Enzyme-catalyzed processing is very specific and can be controlled to produce protein hydrolysates of defined molecular weight distribution, peptide composition, and degree of hydrolysis (DH). Acid and alkali hydrolysis, on the other hand, can destroy L-form amino acids, produce D-form amino acids, and can form toxic substances like lysino-alanine (Lahl and Braun 1994). Enzymatic hydrolysis is developed mostly under mild conditions of pH (6–8) and temperature (40–60°C), avoiding the extremes usually required for chemical and physical treatments and minimizing side reactions (Clemente 2000).

Hydrolysis of proteins by proteolytic enzymes (often referred to as proteases) is a widely used technique to modify the physicochemical properties (e.g., molecular size, surface activity/hydrophobicity, etc.), functional properties (e.g., gelation, emulsification, solubility, etc.) and sensory properties of food proteins. A review article by Panyam and Kilara (1996) provides several examples where limited proteolysis was used to enhance the functionality of food proteins. After the substrate (i.e., protein) and enzyme(s) selection for enzyme-catalyzed processes, the DH is the principal variable to be used in optimization (Alder-Nissen 1986) to achieve desired protein hydrolysates. One of the major elements in commercial production of hydrolysates is developing the optimal enzyme mixture for producing the desired ratio of amino acids, dipeptides, tripeptides, and oligopeptides (Lahl and Braun 1994). The mechanism, key parameters, and enzyme process control method will be elaborated in Sections 72.2 and 72.3.

Although protein hydrolysates can be derived from vegetable- or animal-based proteins, by acid/alkali or enzyme hydrolysis, the focus of this article is on enzymatic-derived hydrolysates from dairy proteins (whey and casein proteins), henceforth referred to as dairy protein hydrolysates (DPH).

There are considerable interests in DPH, driven by advances in technology, economic incentives (value-added products vs. commodity), potential nutritional and health benefits, and market demand. Today, hydrolysis can be performed by conventional batch hydrolysis or by continuous hydrolysis using ultrafiltration (UF) membranes (Korhonen and Pihlanto 2003), thus providing a technological means to develop products with desired peptide composition. Stepwise UF using low molecular mass cut-off membranes may be useful for separating small peptides from high molecular mass residues and remaining enzymes (Korhonen and Pihlanto 2003). Turgeon and Gauthier (1990) used a two-step UF process and were able to produce a mixture of polypeptides and a fraction rich in small peptides with a molecular mass below 2000 Dalton (Korhonen and Pihlanto 2003). Thus, the UF technology is enabling the development of DPH of selective composition that hitherto was unachievable. The range of new product possibilities and revenues resulting from the application of enzymes and UF technology makes protein hydrolysates

a product category of the future. As value-added products, DPH command higher premium ($>2\times$) than commoditized dairy proteins.

Proteins sources used most commonly in nutritional products containing protein hydrolysates are casein, whey protein, and soy (Lahl and Braun 1994). Cow's milk protein is the most important protein source used in the development of protein hydrolysates designed for nutritional support of patients (references in Clemente 2000). They are produced from isolated casein or from whey protein concentrate by using food-grade proteases (Clemente 2000).

Applications for DPH include (1) the development of special formulations designed to provide nutritional support to patients with different needs (Mahmoud 1994; Boza and others 1995; Clemente 2000); (2) infant formulas (Lahl and Braun 1994); (3) as a source of bioactive peptides (Fox and Kelly 2003; Korhonen and Pihlanto 2003); and (4) in protein supplementation for clinical use (Clemente 2000). Several bioactive peptides are present in hydrolysates of caseins and whey proteins. These include phosphopeptides, caseinomorphines and other peptides with morphine-like properties, immunomodulating peptides, blood platelet-modifying (antithrombic) peptides, angiotensin converting enzyme inhibitors and bactericidal peptides (e.g., from lactoferrin) (Fox and Kelly 2003). Much of the knowledge on bioactive peptides is based on bioactivity by *in vitro* assay protocols; however, more research is needed to understand the pharmacokinetics of peptide production and efficacy *in vivo* (Playne and others 2003; Yamamoto and others 2003). The literature on bioactive peptides from dairy proteins has been extensively reviewed by several authors including Fox and Flynn (1992), Mulvihill and Fox (1994), Shah (2000), FitzGerald and Meisel (2002), Pihlanto-Lappala (2002), and Korhonen and Pihlanto (2003).

While the art of manufacturing dairy protein hydrolysates is well understood by food protein ingredient developers, more work is needed to develop cost-effective, industrial-scale technologies that retain and/or enrich bioactive peptides in DPH. The economic value and growth opportunity in DPH and associated bioactive peptides are enormous considering the various nutritional and potential health benefits and the untapped new applications in several functional foods, dietary supplements, and biotechnology.

The purpose and scope of this chapter is to review general aspects of protein hydrolysis, the technology of DPH, functions and applications of DPH, and provide future perspectives.

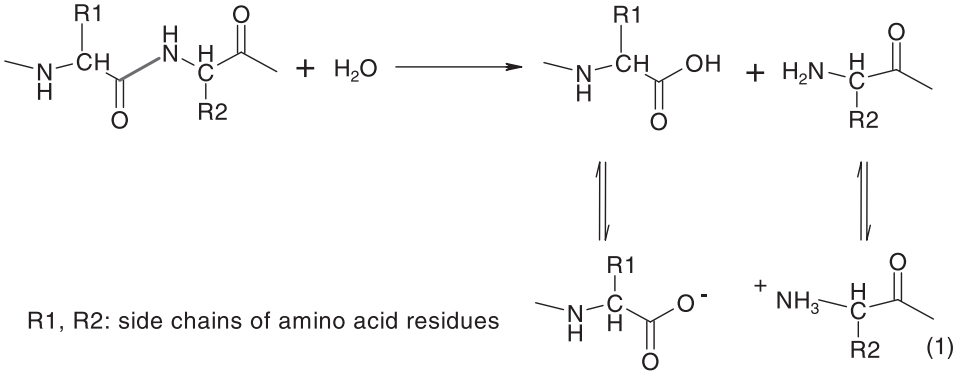
72.2 GENERAL ASPECTS OF PROTEIN HYDROLYSIS

72.2.1 Fundamentals of Protein Hydrolysates

By definition, hydrolysis is a chemical reaction in which a substance reacts with water to be changed into one or more substances. Proteins are made of amino acids linked together linearly by peptide bonds. Hydrolysis of proteins involves the cleavage of these peptide bonds, resulting in breakdown of proteins to peptides and amino acids. The products of protein hydrolysis, which are normally mixtures of peptides and amino acids, are called protein hydrolysates.

Hydrolysis of peptide bonds can be achieved chemically through the use of strong acids or alkalis, or can be achieved enzymatically through the use of proteolytic enzymes (proteases). Equation (1) illustrates an enzyme-catalyzed hydrolysis of a peptide bond. Hydrolysis of each peptide bond generates one free amino group and one free carboxyl

group, which, depending on the pH of the media, can release or take up a proton. At high pH, hydrolysis of peptide bonds results in the release of protons; the up take of protons results at low pH.



Proteases can be categorized into different groups based on their type of action, catalytic mechanism, optimal pH of activity, organisms they are derived from, and so on. The proteases which hydrolyze peptide bonds from the N-terminus or C-terminus, one amino acid residual (sometimes two residuals) at a time, are called peptidases or exopeptidases. The proteases which hydrolyze peptide bonds in the interior of the polypeptide chain are called proteinases or endopeptidases. Based on the mechanism of catalysis, proteinases can be divided into four subgroups: serine proteinases, cysteine proteinases, aspartic proteinases, and metallo-proteinases. Some proteases have a very specific requirement for R1 or R2 to hydrolyze the peptide bond. Such requirements are referred to as the substrate specificity of the protease. For example, trypsin only hydrolyzes the peptide bond when R1 is the side chain of an arginyl or lysyl residue. Therefore, trypsin has a narrow specificity. Some other proteases have broader specificities. An example is Subtilisin A, which hydrolyzes peptide bonds when R1 is the side chain of a hydrophobic amino acid residue.

The rate of protease catalyzed hydrolysis of proteins generally depends on the types of substrates, enzyme and substrate concentrations, pH, and temperature. Increases in temperature generally increase the rate of protease catalyzed hydrolysis until the protease starts to denature and lose its activity. Various proteases have different stabilities to heat denaturation (inactivation). The heat stability of a protease also varies in the presence of different substrate and hydrolysis products. With a given set of conditions, each protease will demonstrate an optimal temperature and optimal pH. The information about proteases' optimal pH and temperature and how they change in the presence of different substrates provide some guidance in developing processes for dairy protein hydrolysates.

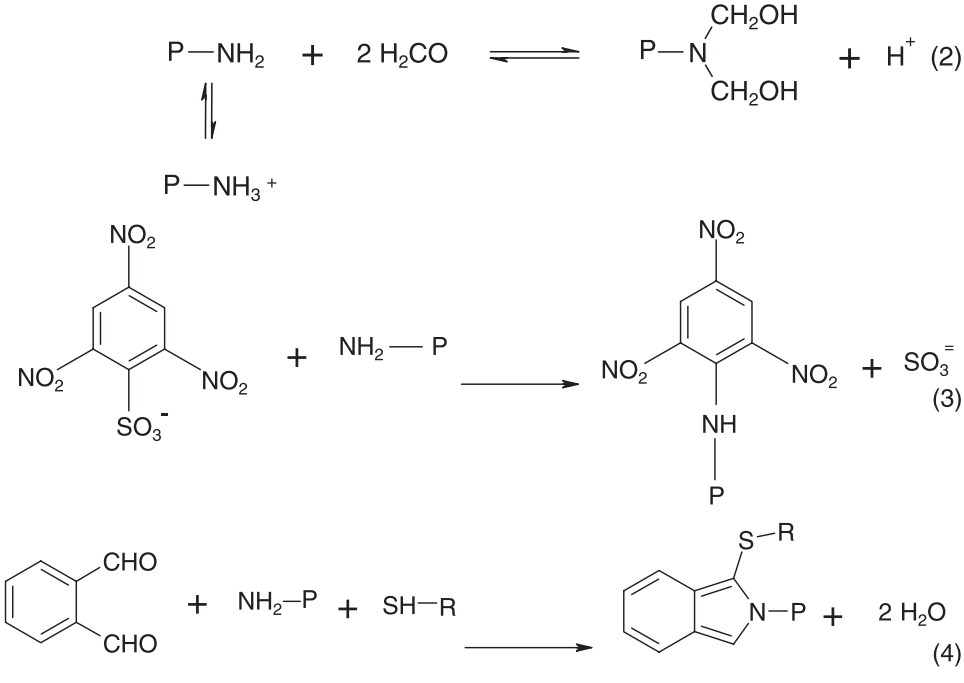
72.2.2 Analysis of Protein Hydrolysates

Depending on the uses of protein hydrolysates, they can be characterized by their level of hydrolysis, peptide profile, functional attributes, and biological activities. Measure of hydrolysis level is the most common form of characterization for any protein hydrolysate. Trichloroacetic acid (TCA) solubility has been used as a measure of hydrolysis level since as a protein is further hydrolyzed, smaller peptides and amino acids, which are more soluble in TCA solution, are formed. Therefore, TCA solubility index has been used as

an indication for the amount of small peptides and amino acids in the hydrolysate products (Margot and others 1994).

Adler-Nissen (1979) has suggested using degree of hydrolysis (DH) as a more quantitative measurement of hydrolysis level. DH is defined as the percentage of peptide bonds hydrolyzed. As shown in Eq. 1, hydrolysis of each peptide bond generates one amino group (and one carboxyl group). By determining the amino groups generated during hydrolysis, one can measure the number of peptide bonds hydrolyzed.

Several methods exist in determining the amino groups in a hydrolysate. Formol titration of amino groups is one of the well-known methods. It is based on the fact that in neutral or alkaline solutions, the amino groups can react with 2 moles of formaldehyde and form a product with much lowered pKa (Eq. 2). If the reaction is carried out at a pH sufficiently lower than the pKa of amino groups, the protons released can be titrated and directly related to the number of amino groups. However, because amino groups in a protein hydrolysate can have different pKa, formol titration usually does not measure all of the amino groups. Reactions of amino groups with trinitro-benzene sulfonic acid (TNBS) or with o-phthalaldehyde (OPA) in the presence of sulfhydryl containing compounds form products which can be measured by absorbance at 340 nm (Eq. 3 and Eq. 4, respectively). These reactions are the basis for the determination of amino groups in protein hydrolysates (Adler-Nissen 1979; Nielsen and others 2001) and ultimately their DH.



Once the amount of amino groups in a hydrolysate is determined by one of the above methods, the ratio of amino nitrogen to total nitrogen (AN/TN) can be readily calculated. AN/TN also reflects the hydrolysis level. For any protein, there is a linear relationship between AN/TN and DH. However, due to the difference in amino acid compositions, hydrolysates from different protein substrates will have a slightly different relationship between AN/TN and DH (Fig. 72.1).

Using size-exclusion chromatography (SEC) on HPLC, the peptide profile (molecular weight distribution) of protein hydrolysates can be determined (Knights 1985; Vijayalakshmi and others 1986; Nakamura and others 1992). Many peptides from hydrolyzed proteins tend to aggregate in aqueous solutions through hydrophobic interaction. When analyzing these hydrolysates by SEC, the mobile phase often contains acetonitrile, trifluoroacetic acid, and salt to disrupt the hydrophobic and ionic interactions. Although HPLC-MS technique can be used to obtain more accurate and specific information about any peptide in a protein hydrolysate, SEC remains the most common technique for general characterization (such as peptide profile) of protein hydrolysates due to its relative ease of operation and availability. Figure 72.2 gives some examples of molecular weight profiles of WPH obtained by SEC on HPLC. The three hydrolyzed whey proteins have calculated DH of 4, 8, and 11, respectively. By proper integration of the areas between different retention times, the percentage of peptide material in various molecular weight ranges can be readily calculated. Another commonly used term to describe the level of hydrolysis is peptide chain length (PCL) (Adler-Nissen 1986). PCL can be directly calculated from DH (Eq. 5) and is a measure of average number of amino acid residues in the peptides. For hydrolysates of dairy proteins, PCL_0 (the number of amino acids residues in a polypeptide chain of an intact protein) is generally in the range of 150–400. Equation 5 will approximate to DH $=$ 100%/PCL (Adler-Nissen 1986).

$$\mathrm{DH} = \frac{\left(\frac{1}{\mathrm{PCL}} - \frac{1}{\mathrm{PCL}_0}\right)}{\left(1 - \frac{1}{\mathrm{PCL}_0}\right)} \times 100\% \tag{5}$$

where PCL_0 is the peptide chain length of the substrate protein.

The need to characterize the functional attributes such as solubility, viscosity, emulsifying properties, and heat stability very often depends on the application of the hydrolysate

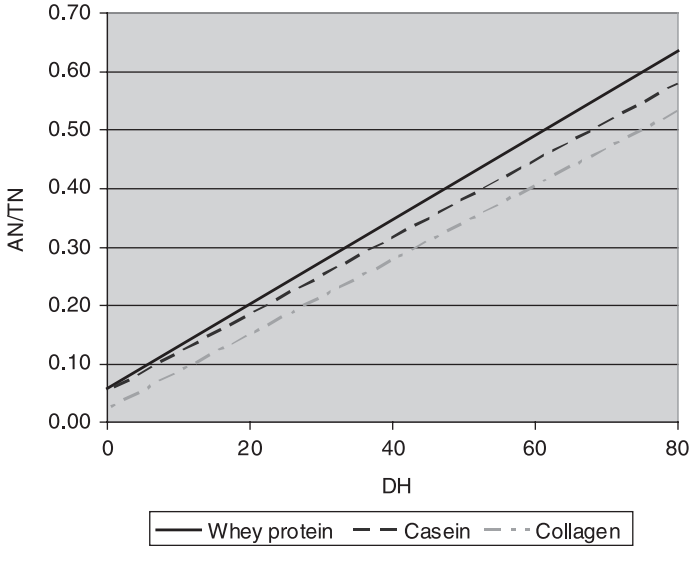


Figure 72.1 *Relationship of AN/TN and DH for hydrolysates of three different types of proteins as calculated based on their amino acid composition.*

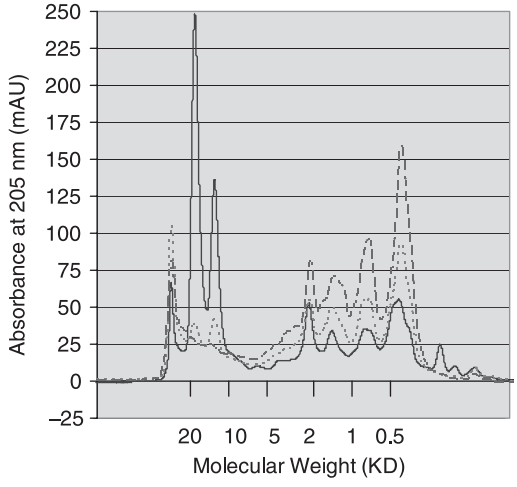


Figure 72.2 *Molecular weight profiles of three hydrolyzed whey protein concentrates with different DHs. The chromatograms were obtained using Tosohass SW2000$_{XL}$ column with aqueous solution of 36% acetonitrile and 0.1% trifluroacetic acid as the mobile phase. Samples: — Hydrolyzed WPC with DH of 4; · · · hydrolyzed WPC with DH of 8; - - - - hydrolyzed WPC with DH of 11.*

products. The techniques used in such characterizations are generally the same as those used to measure nonhydrolyzed protein ingredients (Mahmoud and others 1992; Mahmoud 1994; Boza and others 1995).

In some medical and nutritional applications of protein hydrolysates, the presence of intact proteins and immunoreactive peptides needs to be minimized. Highly sensitive *in vitro* techniques such as enzyme-linked immunosorbent assay (ELISA) and radio allegro sorbent test (RAST) can be used to measure the amount of immunoreactive materials (Otani and Hosono 1987; Asselin and others 1989; Cordle 1994; Van Beresteijn and others 1994). Recently, more attention has focused on various peptides in protein hydrolysates with specific biological activities. Examples include bioactive peptides such as casomorphin peptides, casein phosphopeptides (CPP), angiotensin-converting enzyme (ACE) inhibitory peptides, and so on (Daniel and others 1990; Kitts and Yuan 1992; Schlimme and Meisel 1995). The applications of these bioactive peptides will be discussed later in this chapter. The ability to measure these peptides and/or the bioactivities is often imperative to success of the applications. The measurement of biological activities of protein hydrolysates generally involves enzyme or cell-based assays (Coste and others 1992; Meisel 1997). For instance, ACE inhibitory peptides are measured in an enzyme (ACE) assay using synthetic substrates such as hippnrylhistidylleucine (HHL) or furyllacryloylphenylalanylglycylglycine (FAPGG). ACE catalyzes the hydrolysis of HHL or FAPGG and the activity is measured by the reduction of substrate absorbance or the amount of products formed under standard conditions (Cushman and Cheung 1971; Holmquist and others 1979; Buttery and Stuart 1993). The reduction of ACE activity in the presence of protein hydrolysates reflects the inhibitory activity of the hydrolysates. IC50, the amount of protein hydrolysates which will show 50% of ACE inhibition under the given conditions, is commonly used to represent the measure of the inhibitory activity. Figure 72.3 illustrates data of ACE inhibitor activity assay of two whey protein hydrolysates as compared to the control intact whey protein concentrate.

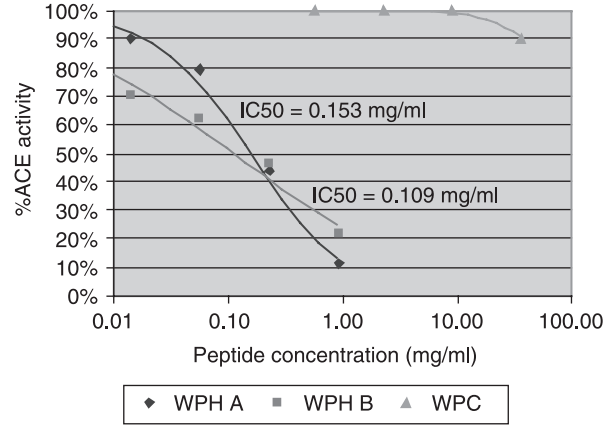


Figure 72.3 *Assay of ACE inhibitor activity of two whey protein hydrolysates and a whey protein concentrate.*

72.3 TECHNOLOGY OF DAIRY PROTEIN HYDROLYSATES

72.3.1 Why Hydrolyze Proteins?

There are many reasons food technologists use hydrolyzed dairy proteins in their food applications. Dairy proteins can be hydrolyzed to modify their functional properties (Panyam and Kilara 1996). For example, hydrolysis of casein can improve its solubility at pH range close to its isoelectric point so that it can be used in applications requiring high solubility in low pH range. Whey proteins, which are normally not very stable to heat treatment in many food processes, can improve their heat stability through enzyme hydrolysis. Much research has been done on the enzymatic hydrolysis of dairy proteins to improve other functional properties such as foaming, gelling, and emulsifying properties (Vojdani and Whitaker 1994; Huang and others 1996; Otte and others 1996). More often, dairy proteins are hydrolyzed for nutritional purposes (Mahmoud 1994; Clemente 2000). Hydrolysis of proteins improves their absorption in our digestive systems (references in Clemente 2000). Hydrolysis is a common approach used to reduce antigenicity and to increase the tolerance of the proteins, especially for infants and patients. Recently, many dairy protein hydrolysates have been produced for the specific peptides or bioactivities released during hydrolysis. Examples of these are CPPs, ACE inhibitory peptides, and so on.

72.3.2 Types of Dairy Protein Hydrolysates and General Production Process

Dairy protein hydrolysates currently fall into three categories based on the types of substrates used: casein hydrolysates, whey protein hydrolysates, and milk protein hydrolysates. With the availability of further fractionated dairy proteins, we can expect to see hydrolysates of fractionated dairy proteins, especially fractionated whey proteins.

Figure 72.4 shows the general process for manufacturing dairy protein hydrolysates. The starting materials are dairy protein solutions or suspensions. They can be reconstituted from dried protein powder and water, or they can be intermediate streams from the dairy protein processes. Examples of the latter are most commonly liquid protein streams before

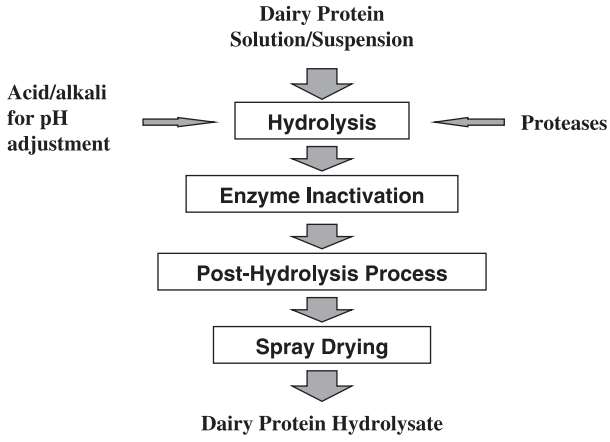


Figure 72.4 *General manufacturing process for dairy protein hydrolysate.*

drying such as liquid whey protein concentrate, milk protein concentrate or converted (neutralized) caseinate solutions.

Although continuous protein hydrolysis in a membrane reaction vessel or in an immobilized enzyme column has been researched (Mannheim and Cheryan 1990; Ge and others 1996), most current commercial operations employ batch hydrolysis carried out in a processing vessel with agitation with the capability to control temperature (heating and cooling), and pH. One of the most critical factors in this step is the selection of the protease or the combination of proteases for a specific substrate. The concentration of the substrate protein, the enzyme to substrate ratio, level of agitation, hydrolysis pH, and temperature are some of the other important factors which will impact the rate of hydrolysis and properties of hydrolysate products. As explained in the last section, protons can be released or taken up depending on pH during protein hydrolysis; therefore, to maintain at the optimal pH for hydrolysis, alkali or acids need to be continuously added. The rate of acid or alkali addition reflects the hydrolysis rate. The total amount of acid/alkali added during constant pH hydrolysis can be used to calculate the degree of hydrolysis (Adler-Nissen 1986).

Because the hydrolysis step in most commercial plants is carried out in batch operation, it is necessary to have an enzyme inactivation step at the completion of the hydrolysis step. The most common way for enzyme inactivation is through heat treatment. The dairy protein substrates still contain some lactose. Reactions of proteins and peptides with reducing sugars such as lactose are promoted at high temperature and are perhaps the most common cause of protein nutritional damage during processing (Hurrell and Finot 1985). The hydrolyzed proteins are more reactive to lactose during the thermal enzyme inactivation step than intact proteins due to increased number of amino groups generated during hydrolysis of the proteins. Therefore, it is undesirable to overheat treat the product during the enzyme inactivation. High temperature-short time (HTST) heat treatment and processing of the product at pH where the proteases are less stable are two approaches that are used to minimize the undesirable side reactions during heat treatment. Not only can different proteases have very different heat stabilities, but a selected protease can show very different stability to heat in a different matrix (substrates, ionic strength, pH, etc.); therefore, the minimal heat treatment to achieve required inactivation very often needs to be experimentally determined. Figure 72.5 shows the effect of different matrices on

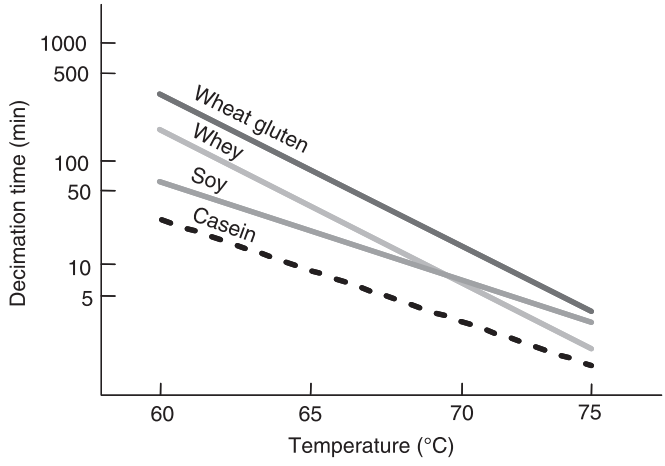


Figure 72.5 *Heat stability of Neutrase in phosphate buffer (pH 6.0) in the presence of various protein substrates. Courtesy of Novozymes North America, Inc.*

the heat stability of a commercial bacterial neutral protease, Neutrase (Novozymes North America, Franklinton, NC). A measure of heat stability of an enzyme is the decimation time (t_D) which is the time required to inactive 90% of the protease activity under a given conditions. Depending on the usage levels of protease in hydrolysis process, heating conditions to achieve more than three to six times of t_D will usually be considered complete inactivation.

Posthydrolysis processes before drying may include many different types of unit operations. These can include a clarification or filtration process to remove insoluble residues, or a fractionation process to separate/isolate peptides of interest and remove peptides with undesirable attributes. Examples of the later include precipitation of certain peptides, membrane separation of peptides based on sizes and adsorption column processes based on various other properties of peptides such as size, charge density, and hydrophobicity.

Sometimes, the hydrolysis is carried out at low protein (or total solids) concentration to achieve the desired attributes of the product. In such a process, the final hydrolysate solution will need to be concentrated through evaporation or membrane processes to maximize the drying efficiency. The drying of concentrated hydrolysates can be carried out by various processes such as roller drying, freeze drying, or spray drying. Spray drying is usually the process of choice for protein hydrolysates due to its efficiency and relatively mild conditions (Lahl and Braun 1994).

72.3.3 Advances in Processing and Analytical Technologies of New Hydrolysate Products

With the advancement of new processing and analytical technologies, it is possible today to develop and produce hydrolysate products with unique properties. An enzyme inactivation step is generally needed in a batch hydrolysis process. Such a process has some inherited limitations especially for proteins which are more heat sensitive such as whey proteins. Therefore, it is difficult to produce whey protein hydrolysate with very low DH in a batch process. Continuous protein hydrolysis processes in an immobilized enzyme column reactor or a membrane reactor not only greatly improves the enzyme

utilization, but also make it possible to produce some bioactive peptides which can be further hydrolyzed and lose activity under batch process conditions (Mannheim and Cheryan 1990; Ge and others 1996). Continuous processing in a column reactor can also produce hydrolyzed whey proteins with very low DH. Such products are reported to have improved gelling, emulsifying, and other functional properties (Chen and others 1993; Huang and others 1996).

Employing various separation technologies, protein hydrolysate products can be fractionated to produce desired peptides. The most commonly used technologies for fractionation of hydrolyzed proteins are membrane processes (Nakamura and others 1992; Pouliot and others 1993). The use of analytical techniques for identifying/quantifying specific peptides, such as HPLC and HPLC-MS, allow the understanding of the kinetics of release and disappearance of the peptides of interest. Such understanding made it possible to develop and produce hydrolysate with specific bioactive peptides (Leonil and Molle 1990; Bouhallab and others 1993). The availability of new commercial proteases with unique specificity also makes it possible to develop hydrolysate products with desired attributes which were previously unattainable. Some of the hydrolysates discussed in the following section are the examples of new generation of protein hydrolysates.

72.4 FUNCTIONS AND APPLICATIONS OF DAIRY PROTEIN HYDROLYSATES

Protein hydrolysates are generally used in enterally administered, nutritionally complete formulas that are given to individuals who have specific nutritional or physiological needs or are used as nutritional supplements for conventional foods (De Wit 1998). Dairy protein hydrolysates have a variety of applications in nutrition (e.g., hypoallergenic infant formula, nutritional/protein supplements, medical foods/enteral formulas), in food (e.g., modifying gelation, emulsion, foam, and flavor characteristics), and in functional foods via bioactive peptides. The term "functional foods" designate foods that provide health benefits beyond nutrition. Functional foods represent an emerging application area for dairy protein hydrolysates with enormous market potential for the health and wellness consumers. It has been indicated that the functional food market is large and in a phase of rapid growth, notably in North America, Europe, and Asia (Playne and others 2003). The global general food market is estimated to be valued at about US$50 billion in 2002 and this market is expected to grow at about 7% per year through 2005 (Sloan 2002). The functional food market in the United States is estimated to be worth about US$18.25 billion, followed by Europe at US$15.4 billion and Japan at US$11.8 billion (Sloan 2002). The global functional dairy food and ingredient market represents a significant segment of this general functional food market, and is also showing strong growth (Playne and others 2003). From a market size of US$4.1 billion in 1998 (excluding Japan market), it is predicted to reach about US$5.3 billion by 2003 (Hilliam 1999; Playne and others 2003). Clearly, dairy protein hydrolysates are functional food ingredients via their potential constituent essential amino acids and bioactive peptides; application opportunities in the functional food market segments are enormous. In the authors' opinion, beverages are uniquely suited as delivery systems for dairy protein hydrolysates. The forecast for functional beverage business growth is strong. According to *Nutritional Business Journal*, functional beverages reached $10.35 billion in sales in

2002, up 10.7%, and are projected to reach $15.9 billion by 2010 (Sloan 2003). Considering the potential opportunities that lie ahead for dairy protein hydrolysates, subsequent discussion in this section will be on emerging new research findings about bioactive peptides from dairy proteins, their potential health benefits and potential applications in functional foods. Also, in this section, key functional properties useful in nutrition products formulations will be discussed.

72.4.1 Selected Functional Properties of Protein Hydrolysates

Functional properties and target applications of dairy protein hydrolysates derived from casein or whey proteins, as with other food protein hydrolysates, are dictated by the extent of hydrolysis, as measured by AN/TN ratio or DH. Mahmoud (1994) suggested a definition for the functional properties of protein hydrolysates to include physicochemical properties that affect the processing, storage stability, organoleptic quality, and nutritional or biological efficacy of the final product. Certain functional properties of the hydrolysates play a more dominant role than others and dictate the choice of the hydrolysate for specific end use (Mahmoud 1994). For example, a hydrolysate targeted for infant formula application will have different process parameters and evaluation criteria than one used for nutritional supplement (e.g., beverage and protein bar applications).

The key physicochemical properties related to functional properties in nutritional products are shown in Table 72.1. Molecular size, commonly designated as molecular weight distribution/profile, has a profound effect on several important functional properties of hydrolysates and thus their application. Osmolality is an important physical characteristic of infant and adult nutritional formulas (Mahmoud 1994). It is a function of the number and size of molecular and ionic particles in the product. In the intact protein-containing

TABLE 72.1 Physicochemical and Functional Properties of Protein Hydrolysates in Nutritional Products.

Chemical and Physicochemical Properties	Functional Properties
Molecular size	Immunogenicity (allergenicity) Solubility Osmolality Viscosity Gelation Emulsification Clarity (turbidity) Flavor
Surface activity and hydrophobicity	Emulsification Foaming
Carbohydrate interaction	Maillard browning Color formation Gelation Flavor formation
Mineral interaction	Solubility Thermal stability

Source: Adapted with permission from Mahmoud (1994).

infant formulas and adult nutritional products, the main determinants of this important physical property are electrolytes, soluble minerals, and simple carbohydrates (Mahmoud 1994). On the other hand, amino acids and peptides contribute significantly to the osmolality of protein hydrolysate-based formulas; the extent of hydrolysis of the protein source in the formulas will directly influence the osmolality of the formulations (Mahmoud 1994). It should be noted, however, that achieving and maintaining proper osmolality in a product is critical to the use of hydrolysates. Those knowledgeable in the art of formulating nutritional products know the importance of optimizing and balancing osmolality in products because solutions of high osmolality (i.e., hypertonic or hyperosmolar products) may draw large quantities of water into the small intestine causing severe diarrhea, possible dehydration, and disruption of electrolyte balance that can induce nausea, vomiting, and abdominal distention (MacBurney and Young 1984; Mahmoud 1994).

Many commercially-available hydrolysates possess good to excellent functional properties in addition to their nutritional attributes. For a detailed discussion on functional properties of hydrolysates, the reader is referred to the article by Mahmoud (1994). For the purpose of this chapter, it is important to note that just as DH dictates the end use of hydrolysates, DH can also be controlled to improve heat stability, emulsification, foaming (overrun), and gelation properties of hydrolysates. An example is the effect of DH on emulsion stability in emulsion-based nutritional products. Because infant formula and specialized adult nutritional products are protein-stabilized emulsions, the emulsifying properties of the protein hydrolysates are important (Mahmoud 1994). Singh and Dalgleish (1998) studied a range of hydrolysates in model system to determine their ability to form and maintain stable emulsions. In their study, commercial hydrolysates of whey proteins with DH ranging from 8% to 45% were used to make emulsions with soybean oil (3% wt/wt); and varied hydrolysates concentrations (0.02–5%, wt/wt). The stability of these emulsions was measured by determining the average sizes of the emulsion droplets and their size distribution both immediately after formation and after storage. The effects of heating on the stability of the emulsions were also determined. As determined by the particle size, the maximum emulsifying capacity was obtained from hydrolysates with a DH of 10% or 20%. Higher hydrolysis resulted in peptides that were too short to act as effective emulsifiers. All of the emulsions were unstable when subjected to heat treatment at high temperatures (122°C for 15 min), but emulsions prepared from less hydrolyzed peptide mixtures were stable to heat treatment at 90°C for 30 min (Singh and Dalgleish 1998).

Perhaps the most important functional characteristic that limits the application of protein hydrolysates is bitterness which is a technological problem attributed to the presence of hydrophobic peptides generated during protein hydrolysis. It is sometimes related to the type of enzymes, DH, and/or protein source. Some proteins such as casein are more susceptible to the generation of bitter peptides than others. Judicious use of exopeptidases in combination with endopeptidases in an optimized ratio has been found helpful to minimize the presence of hydrophobic peptides in hydrolysates (Alder-Nissen 1986; Przybyla 1989; Lahl and Braun 1994; Pedersen 1994; Raksakulthai and Haard 2003).

72.4.2 Application of Dairy Proteins in Selected Nutritional Products

For nutritional products applications, protein hydrolysate composition is generally divided into three major groups: (1) formulas for infants with allergies to intact proteins,

(2) elemental diets for patients with impaired gastrointestinal functions, and (3) nutritional supplements to provide nitrogen in an easily assimilated form (Mahmoud 1994; De Wit 1998). In terms of DH, hydrolysates used in nutritional formulations are generally classified into two broad categories: partially or extensively hydrolyzed proteins (Mahmoud 1994). The extensively hydrolyzed proteins (Table 72.2) have substantially reduced immunological reactivities and are primarily used in hypoallergenic infant formulas (Mahmoud 1994). These proteins are usually comprised of free amino acids and very short peptides. Extensively hydrolyzed protein products are available commercially for both nutritional and food flavor development. Commercial hydrolysates are commonly represented by AN/TN ratio and average molecular weight as shown in Table 72.3. To achieve very low average molecular weight (i.e., high AN/TN ratio) use of whey or casein as protein source often requires an optimized mixture of endopeptidases and exopeptidases for controlled hydrolysis to ensure hydrolysates with low bitterness.

Infant formulas are generally based on milk, whey, soy protein, and protein hydrolysates (American Academy of Pediatrics Committee on Nutrition 1989; Lahl and Braun 1994). Infants who are fed formulas based on cow milk proteins may exhibit allergic reactions to these proteins (Van Beresteijn and others 1994). The most effective means to reduce the allergenicity of cow milk is to decrease the molecular mass of the principal cow milk allergens (namely b-lactoglobulin, a-lactoglobulin, bovine serum albumin, bovine

TABLE 72.2 Molecular Weight Profile of Different Classes of Protein Hydrolysates.

	Degree of Hydrolysis		
Molecular Weight Fraction (Dalton)	Slight/Low	Moderate % in Each Fraction	Extensive
<500	3.8	9.8	90.2
500–1000	1.1	13.5	5.5
1000–2000	1.8	13.7	2.9
2000–5000	3.7	16.6	1.4
>5000	89.7	46.4	0

Source: Adapted with permission from Mahmoud (1994).

TABLE 72.3 Average Molecular Weight and AN/TN of Hydrolysate Products from Casein and Whey Protein Concentrate (WPC).

Hydrolysate[a]	Protein Source	Average Molecular Weight	AN/TN
Intact protein	Casein	28,500	0.07
	WPC	25,000	0.06
Proteose	Casein	6,000	0.13
	WPC	6,800	0.11
Peptone	Casein	2,000	0.24
	WPC	1,400	0.24
Peptides	Casein	400	0.48
	WPC	375	0.43
Peptides and amino acids	Casein	260	0.55
	WPC	275	0.58

[a]Commercial hydrolysates.
Source: Adapted with permission from Lahl and Braun (1994).

serum immunoglobulin G, and the caseins) by enzymatic proteolysis (Van Beresteijn and others 1994). To this end, much research has focused on preparation of hypoallergenic hydrolysates from casein and whey protein. As a result, "first-generation," casein-based hydrolysates have been marketed for more than 40 years for hypoallergenic infant formula applications (Lahl and Braun 1994). These products are characterized by a greater than 70 mol% composition of amino acids and peptides up to eight amino acids long (Lahl and Braun 1994). Second generation, whey protein-based hydrolysates with 40–60 mol% amino acids and peptides up to 12 amino acids long have been marketed for more than 10 years (Lahl and Braun 1994). Third generation, whey protein-based hydrolysates have become available only in the past few years (Lahl and Braun 1994). These formulas have less than 20 mol% amino acids and peptides up to 15 amino acids long (Lahl and Braun 1994; Siemensma and others 1993). Whey protein hydrolysates have been used as a primary nitrogen source in infant formula with reduced allergenic properties (De Wit 1998). To achieve hypoallergenic hydrolysates, ultrafiltration is a necessary prerequisite (Van Beresteijn and others 1994; Ena and others 1995). Whey protein concentrate has been hydrolyzed using the technical food-grade enzyme Corolase 7092 in order to abolish the allergenicity of whey proteins (Van Beresteijn and others 1994). The minimal molecular mass to elicit immunogenicity and allergenicity of whey protein hydrolysates appeared to be between 3000 and 5000 Da, so the molecular weight cut-off value of the filters required must be in this range (Van Beresteijn and others 1994). The amino acid composition of formulas appears to be a critical factor for the nutrition of newborn infants when intact proteins and protein hydrolysates are used (De Wit 1998).

Cow's milk protein is the most important protein source used in the development of protein hydrolysates designed for nutritional support of patients (Clemente 2000). Uses of such hydrolysates include geriatric products, high-energy supplements, weight-control diets, and clinical applications/medical diets for patients with impaired gastrointestinal function either caused by a reduced absorption surface (e.g., short bowel syndrome and Crohn's disease) or by a reduced digestive capacity (e.g., cystic fibrosis and pancreatitis) (Frokjaer 1994; Clemente 2000).

72.4.3 Bioactive Peptides from Dairy Proteins and Potential Applications

Dairy proteins are known to exert a wide range of nutritional, functional, and biological activities that make them suitable ingredients for health-promoting (functional) foods. These properties are partially attributed to physiologically active peptides encrypted in the protein molecules (Korhonen and Pihlanto 2003). These peptides within the sequence of the intact protein can be released by enzymatic hydrolysis during fermentation, controlled enzyme hydrolysis, or gastrointestinal digestion. Bioactive peptides usually contain three to 20 amino acid residues per molecule; the sequence of amino acids defines the function of a peptide fraction (Korhonen and Pihlanto 2003).

Recently, excellent review articles on the subject of dairy/milk bioactive peptides have appeared in the literature (Shah 2000; Floris and others 2003; Kilara and Panyam 2003; Korhonen and Pihlanto 2003). It is clear from these reviews that dairy proteins are rich sources of bioactive peptides with purported physiological activities (e.g., opioid, immunomodulatory, antimicrobial, hypocholesterolemic, antihypertensive, antithrombotic, mineral binding, and so on). Table 72.4 shows a list of examples of physiological active peptides

TABLE 72.4 Examples of Physiological Active Peptides Derived from Casein and Whey Proteins.

Peptide Group/Specific Peptide	Precursor Protein	Physiological Activity
Opioid peptides		
β-Casomorphin	β-Casein	Opioid agonist and ACE inhibition
α-Casein exorphin	α-Casein	
Casoxins	κ-Casein	Opioid agonist
β-Lactorphin	β-Lactoglobulins	Opioid antagonist
α-Lactorphin	α-Lactalbumin	Opioid agonist and ACE inhibition
		Opioid agonist and ACE inhibition
ACE-Inhibitors		
Casokinins	α- and β-Casein	ACE inhibition
Lactokinins	α-Lactalbumin/ β-Lactoglobulin	ACE inhibition
Lactoferroxins	Lactoferrin	Opioid antagonist
Mineral binding peptides		
Caseinophosphopeptides	α- and β-Casein	Mineral binding
Immunomodulatory peptides		
Immunopeptides	α- and β-Casein, α-lactalbumin β-Lactoglobulin	Modulation of immune system
Antimicrobial peptides		
Lactoferricin	Lactoferrin	Antimicrobial
Casocidin	α-Casein	Antibacterial
Kappacin	κ-Casein (glycomacropeptide)	Antibacterial
Antithrombic peptides		
Casoplatelins	κ-Casein	Inhibition of platelet aggregation
Hypocholesterolemic peptides	β-Lactoglobulin	Reduction of total serum cholesterol

Source: Adapted with permission from Korhonen and Pihlanto (2003).

derived from casein and whey proteins. Although, several of these activities are yet to be demonstrated in humans, evidence from *in vitro* and animal studies are encouraging.

A noteworthy novel use of a bioactive peptide derived from casein is the application of casein phosphopeptide CPP in oral care. The calcium-carrier function of caseins has been developed into a functional food ingredient by proteolysis and the extraction of the CPP class of peptides (Playne and others 2003). A commercial food ingredient named Recaldent^{TM} (Bonlac Foods Australia; now Cadbury-Schweppes) fortified with CPP has been demonstrated to protect against the demineralization of tooth enamel (Playne and others 2003; Reynolds 2003). Other examples of commercial products containing bioactive peptides derived from whey proteins or casein via hydrolysis include Biozate 1, cysteine peptide, and lactotripeptide (Ameal S, a dietary supplement), products of Davisco, DMV, and Calpis, respectively. The biozate is a whey protein hydrolysate containing ACE inhibitor peptides, active at 20 g/day in humans. Cysteine peptide is derived from whey protein and is claimed to help in liver detoxification via glutathione enrichment. Lactotripeptide is found in a casein hydrolysate, produced by an extracellular proteinase from *Lactobacillus helveticus* CP790 (Calpis, Japan). The bioactive peptide is

claimed to help maintain a healthy blood pressure and is marketed in the form of fermented milk (i.e., a functional milk beverage) or as an active ingredient in a dietary supplement known as Ameal S. The bioactivity of Biozate 1, cysteine peptide, and lactotripeptide have been tested in animal and clinical trials. ACE inhibitory peptides such as Biozate 1 and lactotripeptide have received more attention in recent years due to the importance of high blood pressure as a major risk factor in cardiovascular diseases.

It is noteworthy to mention the importance of lactoferrin, a minor protein constituent of whey protein. Generally, lactoferrins are single chain polypeptides of about 80,000 Da containing one to four glycans, depending on the species (Spik and others 1994; Steijins and Van Hooijdonk 2000). Bovine lactoferrin consists of about 689 amino acid residues (Steijins and Van Hooijdonk 2000). Lactoferrin not only exhibits both bacteriostatic and bacteriocidal activity against a range of microorganisms (Shah 2000), but also shows significant antiviral activity against both human immunodeficiency virus (HIV) and the human cytomegalovirus (HCMV) (Floris and others 2003), Active peptides named lactoferricin (LF-cin B) can also be derived from it by enzyme digestion (Tomita 1994). Commercial whey protein concentrate will have little ($<0.5\%$) or no lactoferrin. However, owning to advances in chromatography and membrane technology, purified lactoferrin ($>50\%$ purity) products are now commercially available. Lactoferrin and lactoferricin are used in infant formula and dairy foods in Asia. Recently, the Food and Drug Administration approved the use of lactoferrin in fresh meat as a food safety measure. This opens up a new opportunity for lactoferrin-derived products. Using lactoferrin as a starting material, large-scale production of lactoferrin hydrolysate and LF-cin B is readily achievable (Tomita 1994). The product so derived should be uselful as a "a natural" preservative agent in foods and cosmetics and as an ingredient in functional foods.

72.5 FUTURE PERSPECTIVES

The past decade has shown increased interests in the utilization of native and hydrolyzed dairy proteins for human nutrition and health-related foods. The interest in dairy protein hydrolysates and constituent bioactive peptides is expected to remain strong in the future driven by advances in technology, economic incentives (value-added products versus commodity), nutritional and potential health benefits, market demand, and untapped new applications in several functional foods.

While the art of manufacturing dairy protein hydrolysates is well understood by food protein ingredient developers, more work is needed to develop cost-effective industrial-scale technologies that retain and/or enrich bioactive peptides in dairy protein ingredient products. More research is needed in identifying innovative application systems for hydrolysates and bioactive components. Finally, the market success of the future hydrolysate-containing bioactive peptides or isolated bioactive peptides is dependent on the results from well-designed *in vivo* and clinical evaluations.

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73

The Manufacture and Applications of Casein-Derived Ingredients

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73.1 Introduction 558
73.2 Casein Chemistry 559
73.2.1 Molecular Properties of Caseins in Milk 559
73.2.2 Associative State of Caseins in Milk 560
73.3 Manufacture of Casein Derived Additives 562
73.3.1 Hydrolysates 564
73.3.2 Rennet Casein 564
73.3.3 Acid Casein 566
73.3.4 Micellar Casein 568
73.3.5 Caseinates 569
73.3.6 Individual Casein Fractions 569
73.3.7 Coprecipitates 570
73.4 Functional Properties of Casein-Derived Ingredients 570
73.4.1 Solubility 570
73.4.2 Gelation 572
73.4.3 Surface Activity 572
73.4.4 Hydration 575
73.5 Casein Derivatives and Health 577
73.5.1 General Nutrition 577
73.5.2 Specialized Dietary Requirements 579
73.5.2.1 Infant Nutrition 579

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

73.5.2.2 Dietary Requirements of Convalescent Patients 580
73.5.2.3 Lactose Intolerance 581
73.5.2.4 Galactosaemia 582
73.5.2.5 Phenylketonuria 582
73.5.2.6 Hyperuricaemia and Related Conditions 582
73.5.2.7 Sports Nutrition 582
73.5.2.8 Weight Reduction Diets 583
73.5.3 Biologically Active Peptides 583
73.5.3.1 Opioid Peptides 583
73.5.3.2 Immunoregulatory Peptides 583
73.5.3.3 Antihypertensive Peptides 586
73.5.3.4 Mineral Binding Peptides 587
73.5.3.5 Antithrombotic Peptides 587
References 587

73.1 INTRODUCTION

Caseins are a unique group of acidic proline-rich phosphoproteins secreted from the mammary glands of mammals. This chapter will focus on bovine caseins which account for approximately 80% of total protein in bovine milk. The most primary physiological function of casein is to provide a calcium source to the neonate in a form that does not cause pathological calcification in the mammary tissue of the mother. Commercially, caseins are used for their excellent nutritional quality, their distinctive functional attributes, and unique physiological properties. The estimated production volumes and major exporters/importers for casein-derived additives are given in Figures 73.1 and 73.2, respectively. It is the current authors' opinion that only a small fraction, 10–15%, of casein-derived products are used in nonfood applications. This chapter will outline what makes caseins so molecularly unique, illustrate how the different caseins derivatives

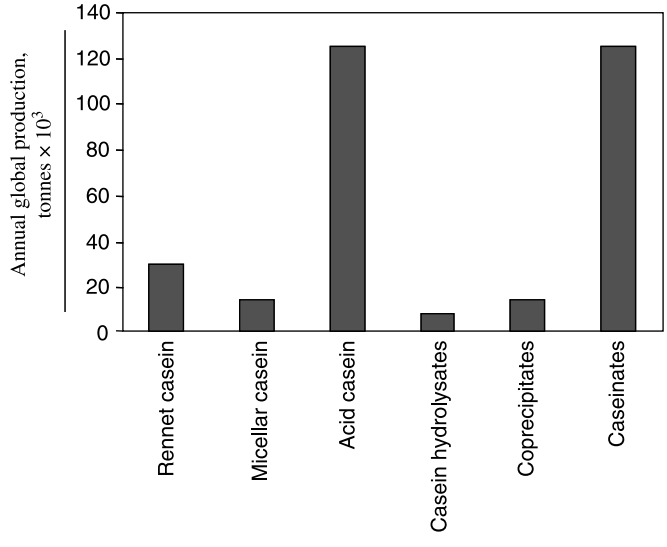

Figure 73.1 *Estimated annual global production of a selection of casein-derived food additives (data from authors).*

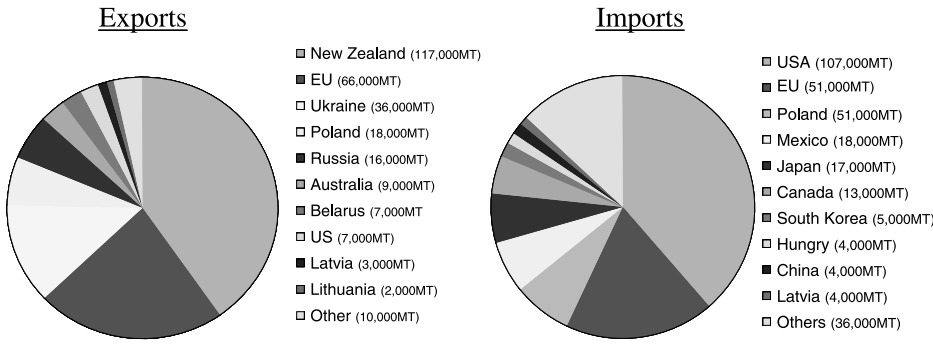

Figure 73.2 *Major exporters and importers of casein-derived ingredients (caseinate and acid and rennet casein). Data taken from USITC Publication 3692.*

TABLE 73.1 References to Reviews on Some Aspects of Dairy Chemistry and Technology.

Topic	References
Casein chemistry	Holt and Sawyer 1988; Swaisgood 1992
Casein micelle structure	Rollema 1992; Holt 1996; De Kruif and Holt 2002
Rennet-induced coagulation	Dalgleish 1992; Tunier and de Kruif 2002
pH-induced coagulation	Roefs 1986
Casein manufacture	Muller 1982; Mulvihill 1992
Drying technology	Kelly and others 2002
Thermal processing	Singh and Creamer 1992; O'Connell and Fox 2002
Uses of caseins	Morr 1982; Southward 1989; Chandan 1997
Specific functional properties	Dalgleish 1997; Dickinson 1997; Dickinson 2002
Nutritional value of caseins	Renner 1983; Hambraeus and Lonnerdal 2002

are manufactured, and discuss the current and potential applications of casein and casein derived products. More detailed reviews on various aspects of casein chemistry and technology are listed in Table 73.1.

73.2 CASEIN CHEMISTRY

Caseins are quite exceptional proteins in terms of the enormous variety of applications. This is due to their unique secondary structure. The true extent of applications for caseins can only be realized when the molecular chemistry of caseins is considered. Indeed, each specific functional or nutritional property of casein or casein-derived ingredients can be directly explained by their physicochemical properties.

73.2.1 Molecular Properties of Caseins in Milk

Bovine caseins can be subdivided into four major fractions: α_{s1}-, α_{s2}-, β-, and κ-casein which account for approximately 35, 15, 38, and 12% of total casein, respectively (Swaisgood 1992). Interestingly, human milk does not contain α_s-casein. Within each casein fraction heterogeneity exists due to genetic polymorphism and posttranslational phosphorylation or glycosylation. κ-Casein is the only casein fraction that is glycosylated.

TABLE 73.2 Some Physicochemical Properties of the Different Casein Fractions.

Characteristic	α_{s1}-Casein (B-8P)[a]	α_{s2}-Casein (A-13P)[a]	β-Casein (A^2-5P)[a]	κ-Casein (B-1P)[a]
Mol. weight, Da.	23,623	25,238	23,988	19,006
Concentration, g/L	10.9	3	9	2.9
Charge at pH 6.6	−21.9	−17.1	−13.3	−2.0
Isoionic point, pH	4.94	5.23	5.14	5.90
Partial specific vol., cm^3/mg	0.725	0.718	0.742	0.734
Hydrophobicity, kJ/residue	4.89	4.64	5.58	5.12
Calcium sensitivity[b], mM	2	3–8	8–15	
Amino acid residues	199	207	209	169
Phosphoserine	8	13	5	1
Lysine	14	24	11	9
$\frac{1}{2}$ Cysteine	0	2	0	2
Proline	17	10	35	20

[a]Genetic polymorph denoted in parentheses (most common polymorph in Western society).
[b]Calcium sensitivity range of all genetic polymorphs within casein fraction.
Source: Swaisgood (1992).

Trisaccharides or tetrasaccharidies may be attached to threonine$^{131/133/135/136}$ or at serine^{141}. Some molecular properties of the genetically-dominant variants of the casein fractions are listed in Table 73.2. The amino acid composition of caseins are not particularly different from globular proteins except for their relatively high proline content and relatively low cysteine or cystine content. The distribution of hydrophobic and charge amino acid residues into specific domains convey an amphiphatic nature to the 1° structure of the casein. This is particularly evident with β- and κ-casein. As a consequence of their distinct amphiphatic nature of their 1° structure, the high concentration of proline residues (which introduce links in the polypeptide back bone) and low content of cystine residues (which act to intermolecularly bridge proteins), caseins do not have a defined 2° or 3° structure. Caseins have been described as "naturally denatured" and rheomorphic proteins (i.e., can adopt different 2° or 3° conformations depending on environmental conditions such as pH, ionic strength, temperature, concentration, etc.). As a consequence of the "loose" 2° structure of caseins and concomitant exposure of hydrophobic domains, caseins readily associate into macromolecular aggregates.

73.2.2 Associative State of Caseins in Milk

In milk, caseins exist as large macromolecular aggregates in colloidal dispersion known as caseins micelles. Casein micelles have a molecular weight of 10^8 Da, a ζ-potential of −10 to −20 mV, a hydrodynamic radius of approximately 100 nm, and a relatively small size distribution (de Kruif 1988; O'Connell and Fox 2001; Tuinier and de Kruif 2002). There are many theories and models as to the structure of the caseins micelles (Rollema 1992; de Kruif and Holt 2002). Generally, a model will, at best, be a simplification of reality. There is consensus, however, that the calcium-sensitive casein fractions, α_{s1}-, α_{s2}-casein, constitute the micelle interior core which is held together by cohesive hydrophobic interactions and calcium-mediated phosphoserine crosslinks. Conversely, κ-casein is predominantly located at the micelle surface, with its hydrophilic C-terminal protruding into the serum phase creating a "hairy layer." β-Casein exists in temperature-dependent dynamic

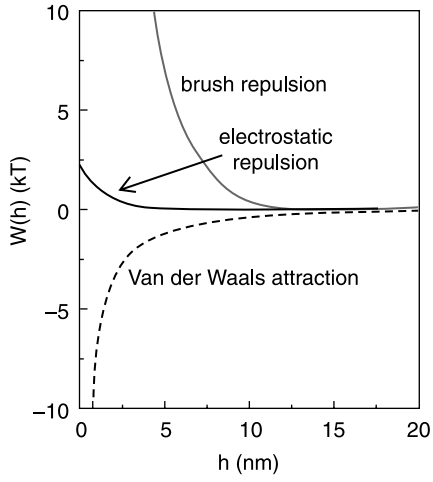

Figure 73.3 *Contributions to the interaction potential between casein micelles at pH 6.7 due to brush and repulsion, electrostatics and van der Waal attractions (Taken from Tuinier and de Kruif 2002).*

equilibrium between the serum and colloidal phases. The principal interactive forces affecting the stability of casein micelles are the van der Waal attractive forces (Eq. 1; Fig. 73.3), polymer brush repulsion forces (Eq. 2; Fig. 73.3) and to a lesser extent electrosatic repulsive forces (Eq. 3; Fig. 73.3). Figure 73.3 shows the relative contribution of the different intermolecular forces on micelle stability.

$$\frac{W_{VdW}{}^{(h)}}{kT} = \frac{-A}{6}\left[\frac{2a^2}{(h+2a)^2-4a^2} + \frac{2a^2}{(h+2a)^2} + \mathrm{Ln}\left\{\frac{(h+2a)^2-4a^2}{(h+2a)^2}\right\}\right] \tag{1}$$

(h: distance separating two micelles; a sphere radius and r is the distance from micelle center to micelle center.)

$$\frac{W_{er}(h)}{kT} = 2\pi a\varepsilon_0 e_r \psi^2 \mathrm{Ln}(1+\exp(kh)) \tag{2}$$

(h: distance separating two micelles; a sphere radius, r is the distance from micelle center to micelle center and Ψ is the surface potential.)

$$\frac{W_{brush}(h)}{kT} = \frac{16\pi a H^2 \sigma^{3/2}}{35}\left(\left(28\left(\frac{2H}{h}\right)^{1/4} - 1\right) + \frac{20}{11}\left(1\left(-\frac{h}{2H}\right)^{11/4}\right) + 12\left(\frac{h}{2H}\right) - 1\right) \tag{3}$$

The "submicelle theory" proposes that casein micelles are made up of hydrophobically-associated submicelles. Submicelles continue to associate, via phosphoserine-calcium crosslinks, until the micelle surface is predominatly located by calcium-resistant κ-casein (see Fig. 73.4). The presence of the "hairy layer" prevents any further association and stabilizes the micelle (Slattery and Evard 1973; Walstra and Jenness 1984).

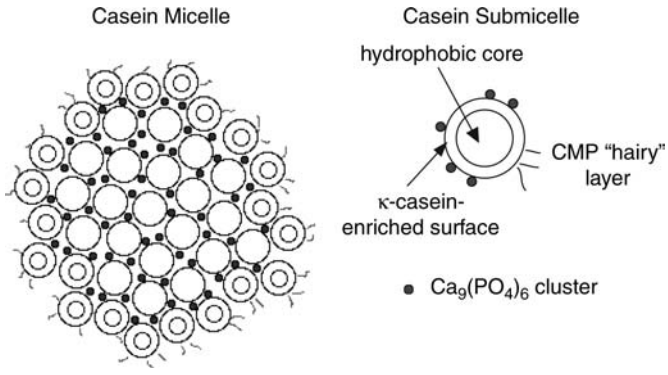

Figure 73.4 *Diagrammatic representation of casein micelle as depicted by the "sub-micelle" theory (from Walstra and Jenness 1984).*

The "nanocluster" theory proposes that calcium-linked phosphoserine residues act as nuclei for the association of casein nanoclusters. Nanoclusters associate until the aggregate surface is predominatly covered with κ-casein, thereby inhibiting any further association (Fig. 73.5; Holt 1992b; de Kruif and Holt 2002).

73.3 MANUFACTURE OF CASEIN DERIVED ADDITIVES

A large variety of casein classes with a milieu of properties exists (Fig. 73.6). Different casein derivatives can be classified according to molecular size or associative state:

- Hydrolysates (glycomacropepetide, surface active hydrolysates, nutritional hydrolysates, physiologically-active hydrolysates)

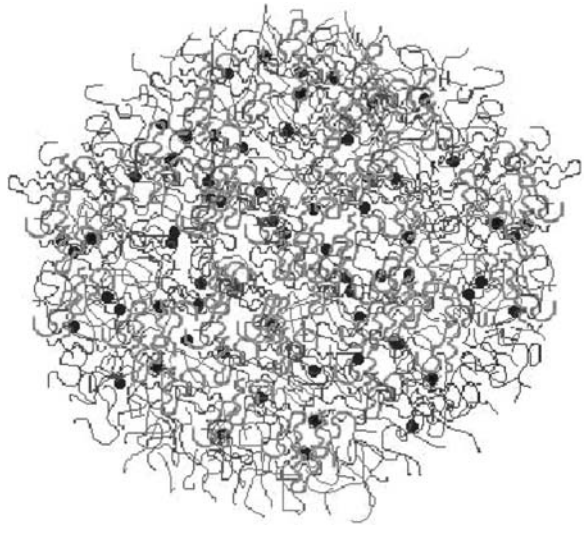

Figure 73.5 *Diagramatic representation of casein micelle structure as depicted by the "nanocluster" theory (taken from Tuinier and de Kruif 2002).*

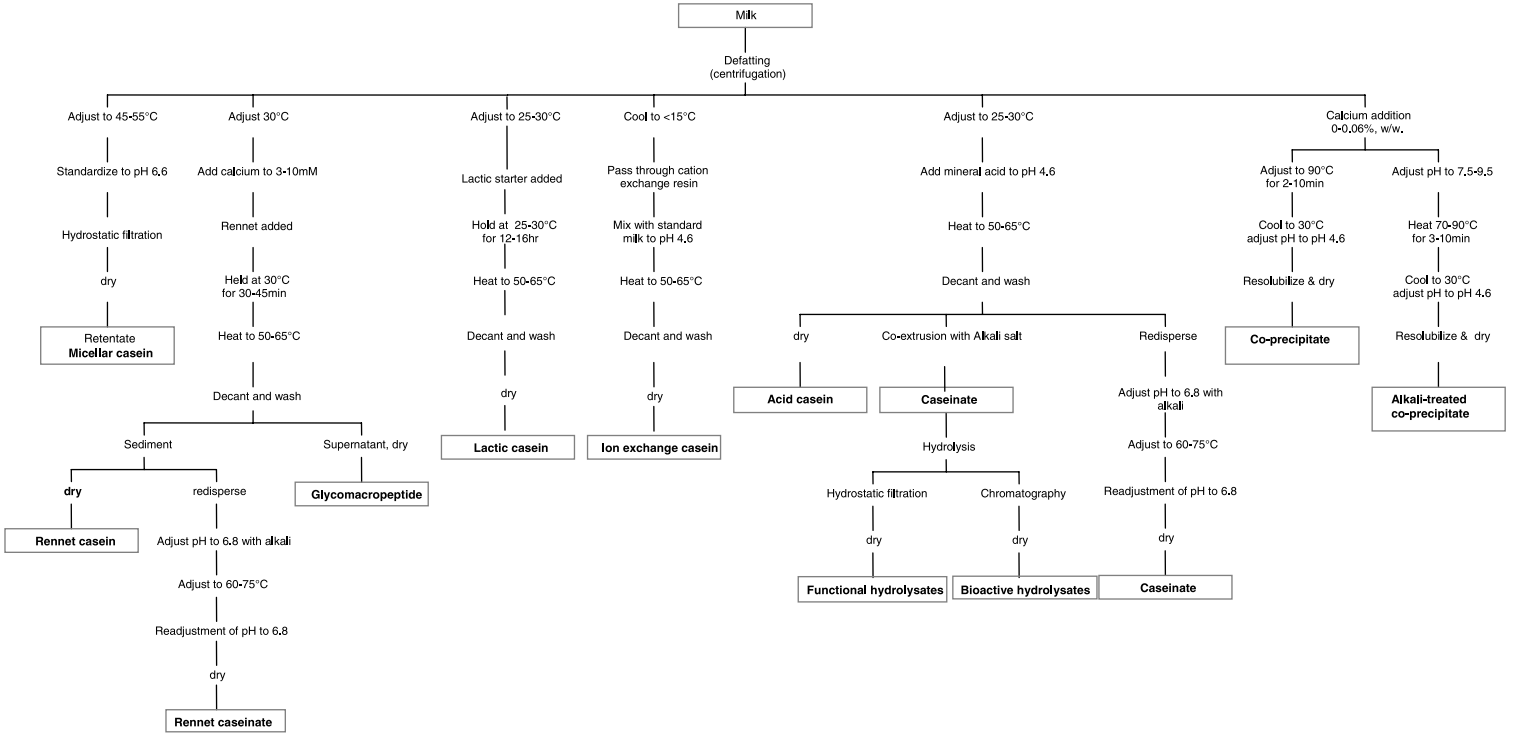

Figure 73.6 *Possible manufacturing processes for a variety of casein classes (data from authors).*

TABLE 73.3 Approximate Degree of Hydrolysis and Free Amino Acid Concentration of Hydrolysates for a Variety of Applications.

Application	Degree of Hydrolysis, %	Free Amino Acid Content, %
Surface active agent	2–12	0
Reduced antigenicity	12–18	Variable
Heat stability/transparency	20	<5
Growth media	30	20–30

- Monomeric caseins (caseinates)
- Colloidal aggregates (micellar casein)
- Macroaggregates (rennet casein, acid casein, coprecipitates)

Regardless of what type of casein-derived product is being manufactured it is imperative that the milk from which it is produced is of good quality. The quality of casein-derived material is affected by the bacterial count of milk, the somatic cell count of milk and the stage of lactation (O'Malley and others 2000). Once delivered to the factory milk is conventionally skimmed and pasteurized. Heat treatments can vary considerably from thermization (60–69°C for 20 s) to pasteurization (LTLT 72°C for 15 s or HTST 85°C for 2 s) to ultraheat (140°C for 1–4 s) treatment.

73.3.1 Hydrolysates

The manufacturing process for casein hydrolysates will differ depending on final use of the hydrolysate. Caseinate or micellar casein is a suitable substrate for functional hydrolysates (i.e., emulsifying agents, foaming agents, reduced viscosity peptides, high transparency-high heat stability peptides), hypoallergenic nutritional peptides or growth media hydrolysates. Downstream processing such as hydrostatic filtration may be used to tailor the molecular weight distribution and free amino acid concentration. Functional or hypoallergenic nutritional hydrolysates are likely to be concentrated by evaporation or reverse osmosis and subsequently dried by spray drying.

The manufacturing process for hydrolysates which are physiologically active or designed for specific nutritional requirements will largely depend on the purity and efficacy the manufacturer aspires to achieve and the quality that the purchaser demands and is willing to pay. At present, hydrolysates which have a low content of active peptides are available (Table 73.3). It should be noted that this area is still in its infancy. As the market matures it is opined that the demand for hydrolysates with a higher content of active peptides will increase. To achieve this end it is likely that more sophisticated fractionation techniques to isolate the substrate or product will have to be used. Non/minimal thermal processing is likely to be employed when concentrating (reverse osmosis, nano/hyperfiltration, freeze concentration) and drying pure hydrolysates (lyophilization).

73.3.2 Rennet Casein

The integral step in the manufacture of rennet casein entails the cleavage of the κ-casein at the Phe^{105}-$Meth^{106}$ sectile bond. Hydrolysis releases the C-terminal glycomacropeptide of κ-casein in the serum phase and consequently destabilizes the casein micelle causing

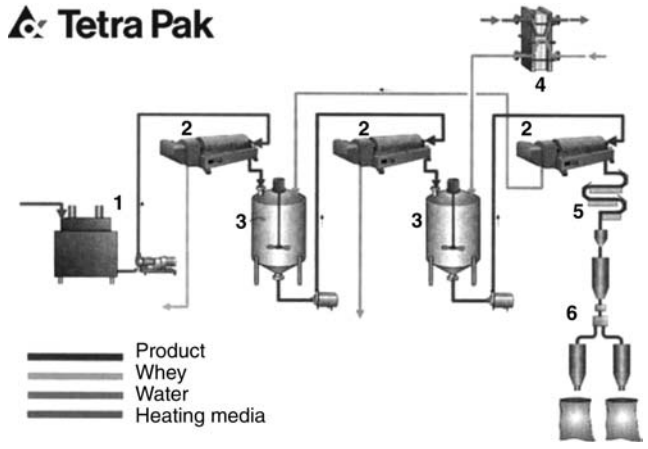

Figure 73.7 *Schematic representation of casein manufacture (1: vat for casein manufacture; 2: decanter; 3: washing tank; 4: drying, milling, sieving, and bagging). (Courtesy of Tetra Pak.)*

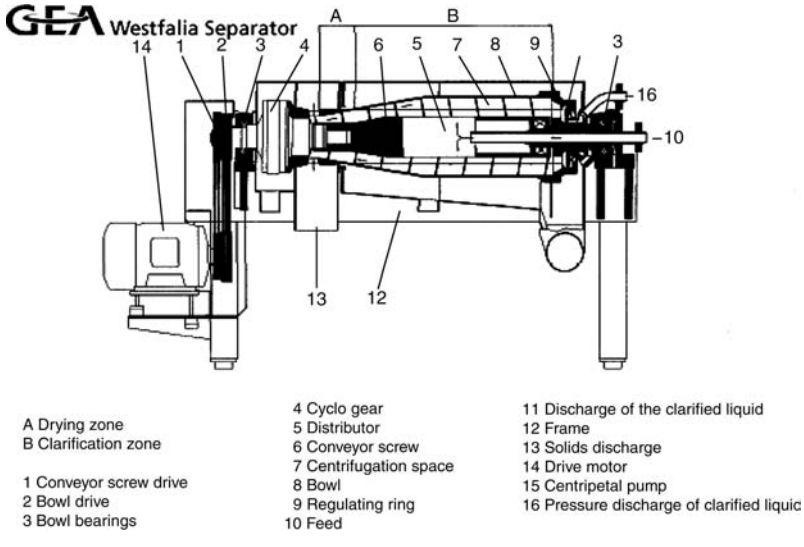

Figure 73.8 *Diagrammatic representation of a centrifugal decanter. (Courtesy of GEA Westfalia Separator.)*

coagulation and gel formation. The enzymes used in rennet casein manufacture are aspartic protinases of animal, vegetable or microbial origin. Once the coagulum has formed it is cooked to 50–75°C and dewheyed. Conventional rennet casein is dewheyed by centrifugal decantation (Figs 73.7 and 73.8). Centrifugal decanters are horizontally-arranged, scroll centrifuges. Feed is pumped using a positive displacement pump into the conico-cylindrical bowl. Under the influence of centrifugal force sediment is deposited onto the wall of the bowl. The casein curd is transported by the scroll while the whey phase is discharged under gravity or with the aid of a centrifugal pump. Rotational speeds for the bowl is in

the order of 4000 rpm with the scroll rotating at approximately 50 rpm faster than the bowl. The curd is then washed to remove residual lactose and whey proteins. Lactose concentrations in the final product should not exceed 0.2% so as to avoid the Maillard reaction and protein crosslinking during drying. Ennis and Mulvihill (1999) reported that the extent of Maillard browning correlated to the hydration characteristics of rennet casein. Residual whey protein can markedly affect the functional properties of rennet casein and should be kept below 2.5%, w/w. Residual lactose and whey proteins, entrapped in the curd matrix, are removed by diffusion through the curd during the washing steps. Countercurrent washing systems are conventionally used and wash temperatures vary considerably between wash cycles (35–75°C). Wash water temperature has a marked effect on the physicochemical properties of the rennet casein properties (O'Sullivan and others 2002). It has been proposed that the effect of wash water temperature is due to whey protein-casein interactions and the concomitant inclusion of whey proteins in the curd (O'Sullivan and others 2002). Generally, a water volume corresponding to 50% of the original milk volume is used during washing. After sufficient washing the casein curd is dewatered; this is achieved by centrifugal decantation producing a curd with a moisture content of 45–55%, w/w. Total casein losses during the dewheying and washing stages should not exceed 1–2% of total casein.

Rennet casein is conventionally dried using an attrition dryer a pneumatic ring dryer (Fig. 73.9*a*), (Fig. 73.9*b*) or a fluidized bed dryer. In ring dryer systems the feed is blown into a high velocity venturi section of the dryer at which stage the bulk of the moisture is flashed off. Conventional inlet temperatures are in the order of 180–250°C. Further evaporation takes place while the sample is being conveyed through the ring duct and enters the manifold where it is classified according to density differences using centrifugal forces. The blade system within the manifold helps to return heavier semidried casein particles back into ring duct for further drying while the dry casein particles are collected using a cyclone system. Typically, curd enters and exits the dryer at approximately 50% and 10–15% moisture, respectively. After ring drying the casein is conventionally milled and/or classified using an impact mill with an air or sieve classifier or fluid energy mill. Some additional moisture loss occurs during milling. Casein is commonly classified according to the USDA ASTM mesh system. Mesh size corresponds to the number of openings across one linear inch of screen. Most common mesh sizes for caseins are 30, 60, 90, and 120 which correspond to openings with a width of about 595, 250, 175, and 125 μm, respectively.

Attrition dryers are also commonly used to dry rennet casein. Attrition dryers are relatively simple units consisting of a mill mounted on a horizontal shaft with bearings on each end. Both halfs of the rotor have static pins while one half has a fixed hammer which generates intense turbulence and concomitant attritional forces. Rotation speeds of approximately 1200 rpm are used, the inlet temperature can be as high as 250°C and residence time is approximately 1–2 s (Muller 1982). Most attrition dryers also have an internal classifier which also grades casein particles according to size and are also fitted with a cyclone or air bag. The most common casein sizes produced using attrition dryers would be correspond to 60, 90, or 120 mesh sizes.

73.3.3 Acid Casein

As the pH of milk is decreases, the caseins' net charge decrease to zero until casein micelles aggregate and a curd is formed at pH 4.6. The pH may be decreased through the addition of mineral acid, *in situ* fermentation (using a mixture of *Lactococcus lactis*

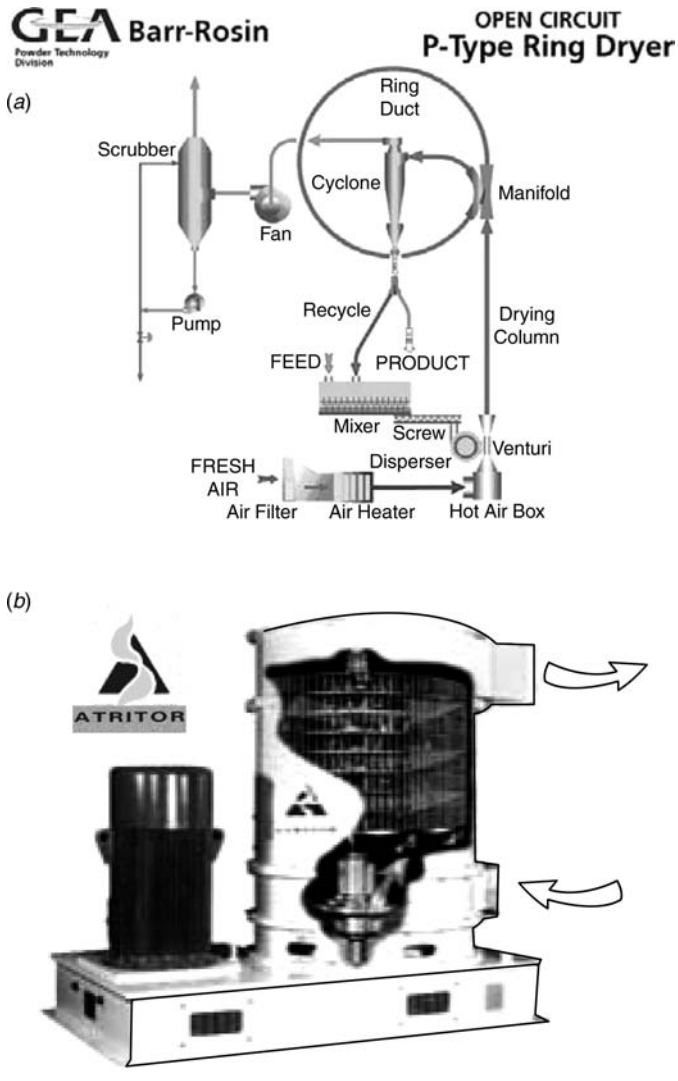

Figure 73.9 *Drawings of ring (a) and attrition (b) dryers. (Courtesy of GEA Barr Roisin and Atritor.)*

and *L. cremoris*), mixing standard milk (ca. pH 6.6) with milk that has been passed through cation exchange resin (ca. pH 2.0; on passing through exchange resin calcium displaces H^+ on the exchange resin with a concomitant release of H^+ into the milk and decrease in pH), or the addition of CO_2 under pressure (CO_2 combines with water to form carbonic acid). Hydrochloric acid is most commonly used since it is relatively inexpensive and produces good quality casein curd. Sulfuric acid is also used to a lesser extent. Acid casein curd is less rubbery than the rennet casein curd, and exhibits a lower tendency to synerese, that is, expel whey (Mulvihill 1992). This is presumably related to the fact that casein micelles in an acid casein curd have a lower tendency to fuse or coalesce. Once the acid curd has formed subsequent treatments are very similar to rennet casein. Great care must be take during the dewheying, washing, and dewateing to remove residual lactose and whey

proteins. Muller (1982) advocated the acidfication of wash water with sulfuric acid so as to reduce wash losses of casein. Casein losses should not exceed 1–2% total casein. Acid casein is conventionally dried using either a pneumatic ring, fluidized bed, or attrition dryer. Epsie and others (1984) reported that acid casein was easier to dry than rennet casein.

73.3.4 Micellar Casein

In the context of this chapter micellar casein is interpreted as casein colloids that are similar in size, surface properties, protein profile, and mineral ion content to native casein micelles in milk. Casein-based, micellar systems such as caseinates and coprecipitates which deviate from these specific characteristics will not behave in a similar manner to native micellar casein from a functional or nutritional perspective.

Tangential hydrostatic filtration which is based on the separation of molecules according to molecular size/associative is a mild, efficient and technologically applicable method for preparing casein micelles. Hydrostatic filtration is classified according molecular weight cut-off of the membrane (Fig. 73.10; Cheryan 1998).

Micellar casein is manufactured by microfiltration. Microfiltration is carried out at temperatures between 20°C and 50°C using spiral-wound synthetic membranes or tubular ceramic membranes and operates at a transmembrane pressure of 1–2 bar pressure. The nominal molecular weight membrane cut off of the membrane is in the region of 0.1–1.0 μm. Casein micelles have a average hydrodynamic radius of 100 nm and as a consequence are retained in the retentate (Section 73.2.2). The retenate is then concentrated by evaporation with or without an ultrafiltration preliminary concentration step. Conventionally, micellar casein is spray dried.

Alternatively, milk protein isolate containing micellar casein and the whey proteins can be prepared by ultrafiltration. Spiral-wound membranes with a nominal molecular weight cut off of 10–20 kDa. are used with the operating temperatures in the order of 20–50°C and a transmembrane pressure of 2–6 bar pressure.

It has also been proposed that micellar casein preparations can be prepared by cryoprecipitation or centrifugaion of milk which has been electrodialysed against acidified whey (see Mulvihill 1994).

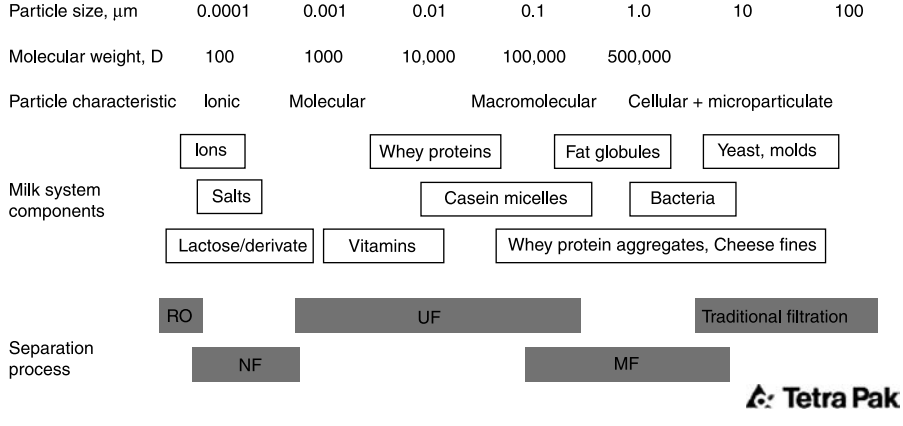

Figure 73.10 *Diagrammatic representation of various hydrostatic filtration processes. (Courtesy of Tetra Pak.)*

73.3.5 Caseinates

Many different forms of caseinate are available, all of which have different functional properties. Caseinates vary with respect to mode of manufacture and the counter ion used. The majority of caseinate is manufactured by mill mixing a minced acid casein curd (50% moisture) with a relatively concentrated alkaline solution. The type of alkali used to manufacture varies considerably. For example, NaOH, KOH, $Ca(OH)_2$, Na_3Citrate, K_3Citrate, NH_3OH are commonly used. The physicochemcal properties of each caseinate class is discussed in Section 73.4. The pH is adjusted to pH 6.6–6.8, and the casein is fully dissolved and dried. Caseinates are conventionally spray dried and to a less degree roller-dried or fluidized bed dried. The general requirements of a spray drier capable of drying caseinate are not very specific. A standard two-stage dryer with integrated fluidized bed would suffice (Fig. 73.11). Centrifugal or hydrostatic atomisation is used. Feed concentrations are generally 25–30%. Inlet and outlet temperatures are in the order of 200–250 and 80–110°C, respectively. Caseinate can also be manufactured by coextruding acid casein with a carbonate (Na_2CO_3 or $CaCO_3$) and then ring or attrition drying.

73.3.6 Individual Casein Fractions

There are a number of reasons why one might wish to prepare individual casein fractions:

- Human milk does not contain α_s-casein and as a consequence there may be some motivation for the development of an α_s-casein-depleted milk protein system for the infant formula industry.
- κ-Casein is a remarkably functional protein with very specific properties. It is quite likely that κ-casein enriched dairy powder preparations would have enhanced physical stability.

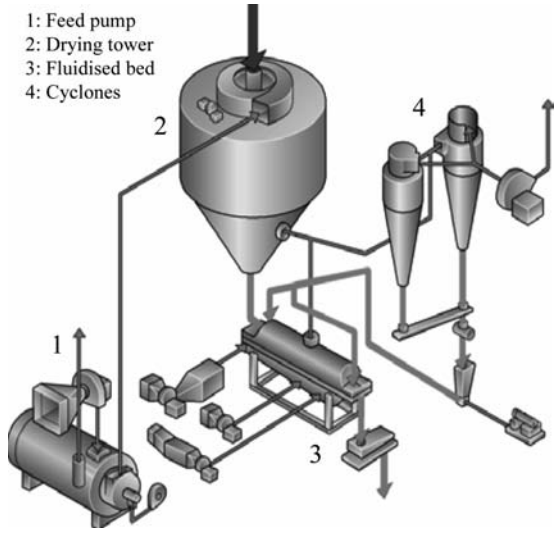

Figure 73.11 *Spray dryer used in the manufacture of caseinate. (Courtesy of GEA Niro.)*

- By isolating individual casein fractions the manufacture of bioactive peptide fractions of high purity may be possible.
- β-Casein is very surface active.

Laboratory scale isolation of individual casein fractions has conventionally used anion exchange chromatography. At low temperatures β-casein readily dissociates as monomers from the casein micelle into the serum phase. The β-casein monomers can then be separated by hydrostatic filtration. Until recently, excellent quality β-casein was available on a commercial scale from a continental European manufacturer.

73.3.7 Coprecipitates

As inferred, coprecipitates consist of casein and whey proteins which are prepared by coprecipitation. Coprecipitation is induced by heat treatment of the milk prior to acidifcation and/or calcification (Table 73.4). Heat-induced covalent crosslinking between the whey proteins and κ-casein through sulfydryl–disulfide interchange reactions play an integral role in coprecipitate manufacture.

73.4 FUNCTIONAL PROPERTIES OF CASEIN-DERIVED INGREDIENTS

The following section deals with the current and potential applications for casein and casein-derived products. The principal factor which qualifies casein as an excellent food ingredient is its pleasant, neutral taste, and soft texture which is neither gritty nor rubbery. This chapter is structured in such a way that functional properties of caseins in general are discussed and specific examples are listed. It should be noted that this list is by no means assumed to be the definitive directory of casein applications (Table 73.5).

73.4.1 Solubility

As solutes, caseins and casein-derived products are a very versatile group of food additives. Caseinates are soluble to high concentrations (20–30% solids) at pH values on the alkaline or acidic side of their isoelectric point. Na-/K-caseinate form translucent solutions which are very heat stable at pH 6.5–7.0 (easily tolerate 140°C for 1 h). The voluminosity of Na caseinate is in the order of 6–9 mL/g (Boulet and others 1998). At acidic pH values Na-/K-caseinate forms thick viscous gel-like systems. Ca-caseinate forms an opaque solution at neutral pH and is not as heat stable as Na- or K-caseinate (Moughal and others 2000). Standard coprecipitates are sparingly soluble in water while variants prepared by heat treating at alkaline pH values are markedly more soluble. In general, caseinates exhibit pseudoplastic rheological behavior but have been

TABLE 73.4 Types of Milk Protein Coprecipitate.

Class	Heat Treatment	$CaCl_2$, %	Acidification	pH
High	90°C × 1–2 min	0.2	No	5.8
Medium	90°C × 10–12 min	0.06	Yes	5.3–5.6
Low	90°C × 15–20 min	0.03	Yes	4.6–4.8

Source: Mulder (1982).

TABLE 73.5 Principal Applications for Casein-Based Products in the U.S. Market.

Product	Processed Cheese	Other Dairy	Speciality Nutrition	Nondairy Foods	Bakery	Animal Feed	Other
MPC 40–49	7%	64%	2%	17%	7%	3%	0%
MPC 50–59	6%	45%	38%	0%	12%	0%	0%
MPC 70–79	95%	2%	0%	3%	0%	0%	0%
MPC 80–89	22%	1%	77%	0%	0%	0%	0%
MPC 90+	0%	0%	95%	0%	0%	0%	0%
Acid casein	11%		25%	60%	1%	<0.5%	2% (Industrial)
Rennet casein	16%	5%	0%	79%	0%	<0.5%	0%
Total caseinate	3%	12%	73%	9%	<0.5%	1%	2% (meat)
Na caseinate	5%	16%	59%	15%	2%	1%	4% (meat)
Ca caseinate	1%	5%	94%	0%	<0.5%	0.5%	0%
Hydrolysates	0%	27%	48%	3%			21% (growth media)

shown to be thixtropic at high shear rates (Mulvihill 1994). The viscosity of Na caseinate solutions is affected by pH, calcium concentration, temperature, and concentration (Fig. 73.12). Soluble caseinates[1] impart a full and satisfying sensation with a clean aftertaste. Caseins are added to coffee creamers, dips, cream liquors, and many other products for their distinctive solubility characteristics amongst other reasons. Rennet casein is insoluble in water below pH 9.0. Phosphates (disodium orthophosphate, disodium pyrophosphate) and/or citrate (trisodium citrate) salts (commonly but incorrectly referred to as emulsifying salts) are often used to solubilize rennet casein during the manufacture of analog and processed cheeses (Ennis 2000). The principal application for rennet casein is in the manufacture of analog and processed cheese products. The main role of rennet casein is to emulsify fat and form the continuous matrix which provides a very characteristic stretch to the final product when cooked. It remains unclear which physicochemical properties are responsible for the stretch of analog cheese.

The development of casein hydrolysates as specific protein solutes has received a lot of research attention recently (Kilara and Panyam 2003). Hydrolysates may be tailored to produce highly transparent and heats stable solutions at acidic pH values (e.g., soft drink applications), or very low viscosity protein solutions (e.g., intravenous nutrition).

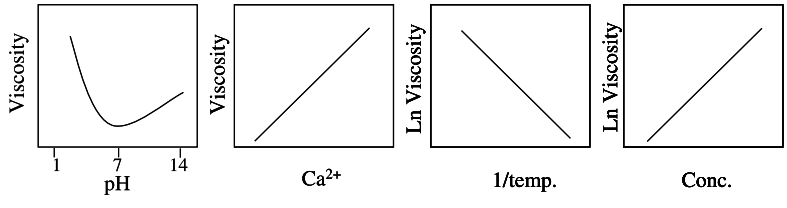

Figure 73.12 *Schematic representation of effect of pH, calcium, temperature and concentration on the viscosity of Na-caseinate solutions.*

[1] Caseinates refers to caseinates and also acid caseins which are resolubilised prior or dring the manufacture of the final product.

73.4.2 Gelation

The gelation of casein in the manner of cheese manufacture dates back to antiquity. Nowadays casein-derived additives are used in many diverse ways to modify gelation properties and/or induce gelation *per se*. The use of micellar casein as an alternative to skimmed milk powder for the standardisation/supplementation of cheese milk has been advocated. The advantage of micellar casein over skimmed milk powder is that there is not a concomitant increase in whey solids that have to be subsequently treated. The application of micellar casein in the development of low-carbohydrate gelled dairy products such as yogurt-like products also warrants research. It has been demonstrated that yogurts prepared using micellar casein have a firmer texture than yogurt prepared using skimmed milk. This is presumably related to the fact that the nonmicellar casein milk constituents, (i.e., whey proteins and lactose) interfere with the continuous gel matrix. Yogurt-like products prepared using micellar casein have to be supplemented with lactose (ca. 0.7%, w/v) to facilitate microbial fermentation. It is opined that the addition of lactose to 0.7%, w/v, would suffice. The ability of mixtures of casein (micellar casein or caseinates) and specific biopolymers (carrageenans and pectin) to interact and gel has been studied extensively (Lynch and Mulvihill 1992, 1994). In our laboratory extensive research has been carried out in the development of low-carbohydrate dessert gels containing mixtures of micellar casein and biopolymers. Acid-induced gelation of caseinates as heterogeneous or mixed systems has also been extensively researched (Dickinson and Casanova 1999; Koh and others 2002). Na-caseinate-gelatin mixtures gel when heated to 30–40°C and redissolve when cooled. Hydrophobic bonding is the principal associative mechanism.

73.4.3 Surface Activity

Emulsions are essentially two phase systems with oil globules dispersed in a continuous aqueous system or *vice versa*. A foam is a system in which air bubbles are dispersed in a continuous aqueous system. Density differences between the aqueous and oil/air phases drive phase separation which is affected by temperature, viscosity and globule/bubble size amongst other factors (Eq. 4).

$$V = \frac{2a^2(\rho_{sol} - \rho_{disp})g}{9\eta} \tag{4}$$

(V: creaming/sedimentation velocity; a: globule/bubble diameter; ρ_{sol} and ρ_{disp} are the density of the continuous and dispersed phases, respectively. g: gravitational constant. η: viscosity of the continuous phase.)

As shown in Eq. 4 globule/bubble size has a marked effect of the rate of creaming or sedimentation. As a consequence, it is imperative to stabilize the globules/bubbles while relatively small and also to prevent subsequent coalesence or growth of globules/bubbles. Surface active agents are amphiphatic molecules (i.e., have distinct hydrophilic and hydrophobic moieties) of varying size and composition capable of decreasing the surface tension at a water-oil or water-air interface and preventing the growth of globules/bubbles. In order for an additive to form and stabilize an emulsion or foam a number of properties are required:

- Nontoxicity;
- Thermodynamic compatability with other constituents in system;

- Sufficient molecular mobility to get to the interface before coalescence of the emulsion or collapse of the foam;
- Capablity to decrease the surface tension at water-air/oil interface;
- Contribution to long term stability at interface.

Casein-derived products are used as emulsifiers and foaming agents in many different food systems. Most low molecular weight surfactants such as phospholipids, mono/diglycerides, sucrose esters, lactylates, and so on, have very clearly defined hydrophobic and hydrophilic moieties. Conversely, casein-derived surface active agents, like all proteinaceous surfactants stabilize interfaces by extending their hydrophilic or hydrophobic moieties into the aqueous or oil/air phases, respectively. Because of their rheomorphic tertiary structure, caseins can easily adapt into the most entrophically stable state (i.e., state of least energy with hydrophilic and hydrophobic amino acid side chains protruding into the aqueous and oil/air phases, respectively). Figure 73.13 illustrates the specific stabilising mode of low molecular weight surfactants, globular proteins and caseins. The stabilizing mode of casein-derived additives is intrinsically linked to the molecular weight of the additive in question. In the current communication we tentatively categorize casein-derived surfactants into three groups (1) hydrolysates, (2) monomeric surfactants, that is, caseinates, acid casein, and (3) colloidal systems, that is, casein micelles. The different molecular weight of each species means that each type will differ with respect to their ability to move to the interface, decrease the surface tension and stabilize the system. Casein hydrolysates are commonly used in conjunction with low molecular weight surfactants to stabilize emulsions and foams. Because of their low molecular weight peptides rapidly migrate to the interface to decrease the surface tension and stabilize the system. Casein hydrolysates are particularly suitable surfactants because of their ability to stretch along the interface and also due to their tendency to engage in hydrophobic associative interactions and hydrogen bonding. Casein hydrolysates are used in a variety of products including ice cream, beverages, nougat, and aerated deserts.

Monomeric casein molecules in the form of caseinates are widely used as emulsifying and foaming agents. Caseinates stabilize interfaces in a manner similar to casein hydrolsates but because of their higher molecular weight are less molecularly mobile and do not migrate to the interface as rapidly as hydrolysates. However, once at the interface caseinates stabilize the interface very well. Enhanced stability is presumably related to the fact the more proteinaceous material has aggregated at the interface which in turn increases the composite density of the oil droplet/air bubble (Eq. 5).

$$\rho_{\text{composite}} = (r^3\rho_{\text{disp}} + [(r+\delta)^3 - r^3]\rho_{\text{inter}})(r+\delta)^3 \tag{5}$$

(r: radius; ρ_{disp} or ρ_{inter}: density of dispersed phase or interfacial material, respectively, δ: interface depth.)

The presence of aggregated protein at the interface leads to the formation of a multilayer structure with a resultant increase in the surface dialational viscosity (Eq. 6) forming a cohesive structure at the interface. The relationship between the associate state of a surface active agent and surface tension is expressed by the surface dialational modulus (Eq. 7). Caseinates are used as emulsifying and foaming agents for many products such as cream liquors, coffee creamers, communited meat products, processed cheese, confectionary, and bakery products. The foaming ability of caseinate is dependent

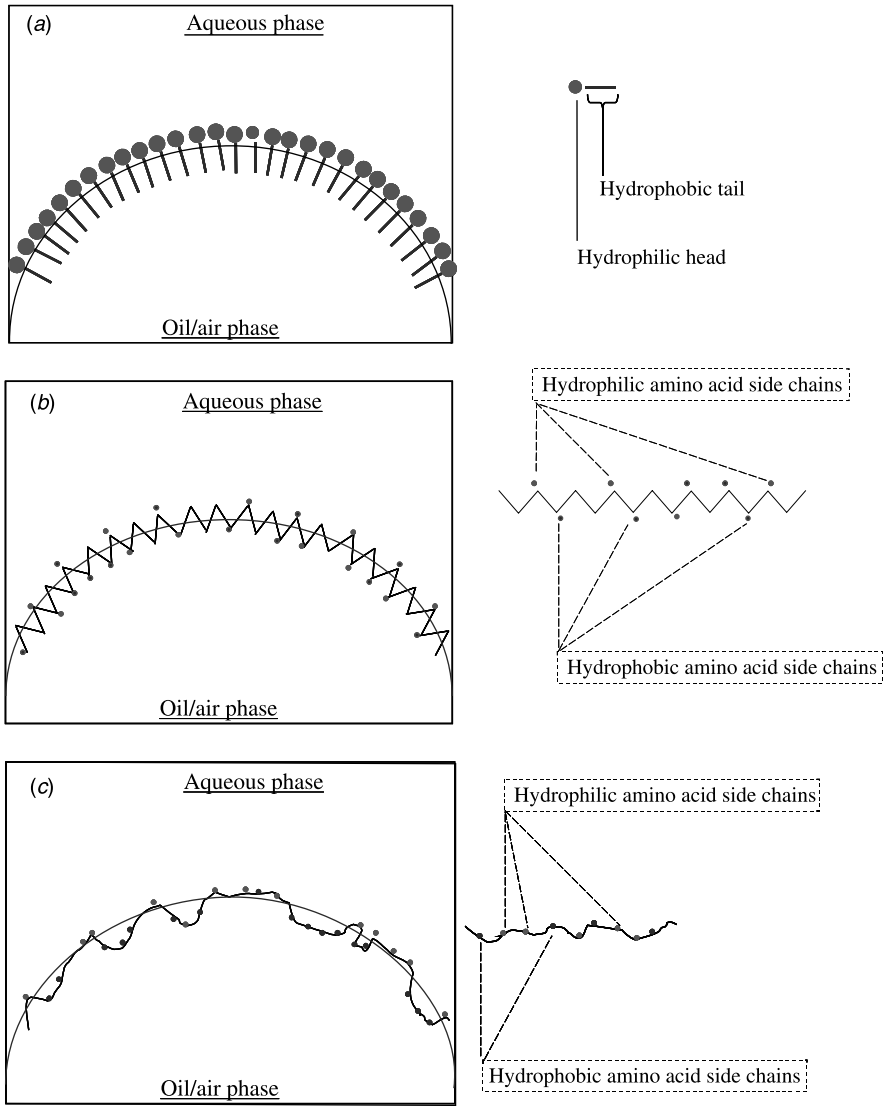

Figure 73.13 *Schematic representation of alignment of low mol. weight surface active agents (a), globular proteins (b), or caseins (c) at a water-oil/air interface.*

on ionic strength, calcium concentration, pH, and so on (Fig. 73.14; Mohanty and others 1984). It would appear that in Na caseinate systems monomers migrate to the interface (Fang and Dalgleish 1993) while in Ca caseinate systems, aggregates move to the interface (Srinivasan and others 2001). As

$$\eta = \Delta\gamma(\mathrm{dLn}\,A/\mathrm{d}t)^{-1} \tag{6}$$

(η: surface dialational constant; γ: surface tension; A: surface tension, and t: time.)

$$\varepsilon_d = \frac{1}{2}(d\gamma/dLnr) \tag{7}$$

(ε: surface dialational modulus, γ: surface tension, and r: radius.)

Casein micelles, in the form of skimmed milk powder or as purified micellar casein systems are widely used as emulsifying and foaming agents. The larger hydrodynamic size of casein micelles (200 nm) (O'Connell and Fox 2001) compared to monomeric casein units (e.g., β-casein ca. 8 nm) (O'Connell and others 2003) means that casein micelles move to the interface very slowly, but it as been shown that micellar systems confer high stability to emulsions. Examples of the use of micellar casein to stabilize emulsions include ice cream (3–5%), dairy whiteners (2–15%), cream liquors, beverages (0.5–3.0%), frozen deserts (0.2–1.0%), and mousses (1–5%), and so on. Chang and others (1995) reported that light hydrolysis of micellar casein markedly improved low fat ice cream.

73.4.4 Hydration

The tendency of caseins and casein-derived additives to bind water is of great significance to many applications. In general caseins are highly hydrated proteins. Interestingly, the water-binding ability of caseins varies considerably within casein fractions (Hong and others 1997):

$$(\text{Hydration } \beta\text{-casein} \gg \alpha_{s1}\text{-casein} > \alpha_{s2}\text{-casein} > \kappa\text{-casein})$$

Na caseinate binds water more readily than Ca caseinate with the differences being more pronounced at high pH and high A_w values (Rüegg and Morr 1984). Rüegg and others (1974) reported that micellar casein is more hydrated than rennet casein. Diamante and others (1992) recorded the sorption profiles of rennet, lactic, and mineral casein. All three casein variants had sigmoidal type II sorption isotherms according to BET classification and had hysteresis loops in their water sorption profiles (Fig. 73.15). Hysteresis loops are common features of sorption profiles of protein systems and have been attributed to the structural rearrangements of the protein upon the uptake of water. The equilibrium moisture content of caseins differed significantly. The following order in extent of

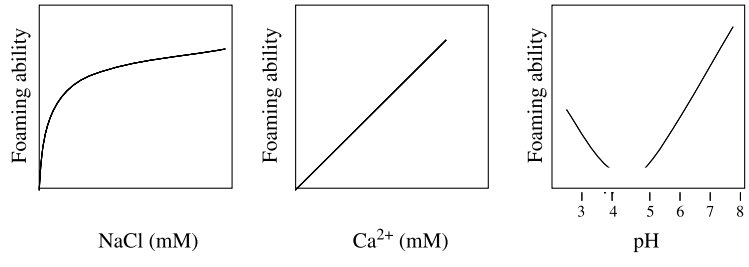

Figure 73.14 *Diagrammatic representation of effect of NaCl, calcium, and pH on the foaming ability of casein.*

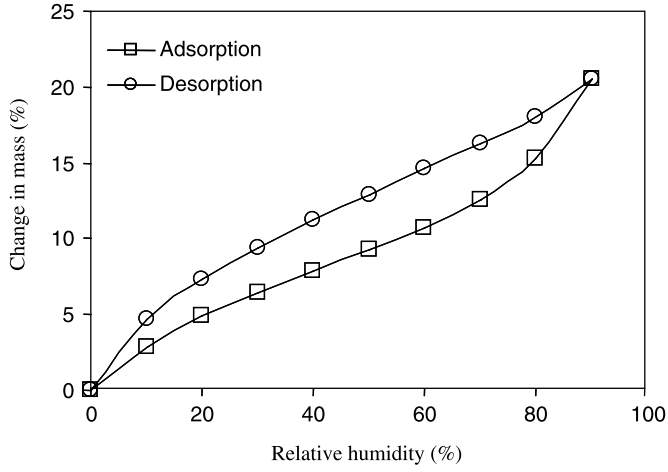

Figure 73.15 *Diagrammatic representation of hysteresis loop in the sorption profile of acid casein (O'Connell and Flynn, unpublished data).*

hydration was evident:

(Mineral casein < rennet casein lactic casein)

Rennet and acid casein differ considerably compositionally (acid casein has much lower mineral content while rennet casein has lost the hydrophilic C-terminal of κ-casein in the form of the GMP) which explains the why rennet casein has a low water binding capacity compared to acid casein. An explanation for the low water binding ability of lactic casein is less obvious but may be related to the fact that starter culture cells are incorporated in the casein matrix. The monolayer moisture content of different casein variants correspond to their overall water binding ability (Diamante and others 1992) which implies that compositional differences rather than structural arrangements are responsible for the different equilibrium moisture content (EMC) of casein variants. Processing factors also affect the water binding capacity of caseins. Espie and others (1984) investigated the effect of milk pasteurization (72 vs. 78°C), precipitation pH (pH 4.50 vs. 4.75), cook temperaure (45, 50, or 55°C), wash water ratio (0.3–0.5), hot wash temperature (60 vs. 80°C), drying mode (no drying, lyophilization, or pneumatic ring drying), or drying temperature (150, 180, or 220°C) and concluded the EMC of casein was only significantly effected by the latter two factors. O'Sullivan and others (2002) also reported that increasing drying temperature within the range of 80–140°C increased the hydration time of rennet casein when dispersed in a disodium orthophosphate solution and attributed this to differences in the fracturabiity of the powders rather then any heat-induced chemical changes.

Voluminosity studies of casein micelles have reported hydration values in the order of 4–6 mL/g protein. Sorption studies report hydration values of caseinates in the order of 0.4–0.6 g H_2O/g protein (Curme and others 1990; Mistry and others 1990). Caseinates and soluble coprecipitates are used as water binding agents in an array of products. Caseinates are added, at of 1–10%, w/w, to communited meat products to emulsify, gel, and bind water. Casein products are also added to whole meat joints, such as cured hams, through brine injection. Pasta formulations containing from 1% to 30% casein have

also been developed to enhance the nutritional quality, freeze-thaw stability, and water binding ability of this food. The performance of casein-derived additives in bakery applications is also markedly dependent on their water binding ability. It should be noted that the water binding ability of a milk protein preparation will depend on a number of factors including (1) protein composition (pure casein vs. whey-casein mixture), (2) predrying processing (heat treatment), (3) drying mode (ring dryer vs. spay dryer) and many other factor such as storage time/conditions, food matrix, formulation conditions, and so on.

There are also applications for casein preparations which have little tendency to bind water, that is, coprecipitates. Breakfast cereal or biscuit formulations containing hydratable proteins become soggy when mixed with water/milk. Conversely, breakfast cereal prepared with insoluble casein coprecipitates remains crunchy when mixed with water/milk, and so on. Related applications include frostings for donuts, nutrition bars, and aerated confectionary products. When selecting suitable coprecipitates it is also important that the product has the appropriate sensory attributes as some coprecipitates have very distinctive and deleterious Maillard and disulfide sensory undertones.

73.5 CASEIN DERIVATIVES AND HEALTH

The recommended daily protein intake is thought to be approximately 0.6–0.9 g/kg body weight (Renner 1983; Manninen 2002). In Western society, it is estimated that milk proteins account for up to 20–30% or 55–65% of total or animal protein intake, respectively (Renner 1983; Hambraeus 1992). The quality of casein and casein-derived products in meeting general and specific nutritional requirements is discussed and some proposed salutary health effects of casein-derived biologically active peptides are introduced (Fig. 73.16).

73.5.1 General Nutrition

Qualifying the nutritional value of proteins is a complicated process as one has to consider the nutritive value of protein in relation to many factors:

- Growth requirements
- Maintenance requirements
- Nitrogen content of protein
- Biological availability
- Value of a particular protein within a certain basal diets.

As shown the amino acid requirements of humans vary considerably (Table 73.6), as does the nitrogen and amino acid contents of different proteins (Table 73.7 and Table 73.8). The amino acid profile of casein is relatively similar to most proteins except for the comparatively low concentration of cysteine residues. The ratio of essential amino acids to nonessential amino acids of milk protein, egg protein, and soy protein is 3.22, 3.20, and 2.58, respectively (Blanc and Sieber 1978). Within the casein fractions β-, α_s-, and κ-casein contain 48.4, 43.3, and 41.1 g essential amino acids per 100 g protein, respectively (Renner 1983). The biological availability of casein (protein digestibility-corrected amino acid score (PDCAAS = 1) is very high compared to meat (PDCAAS = 0.92),

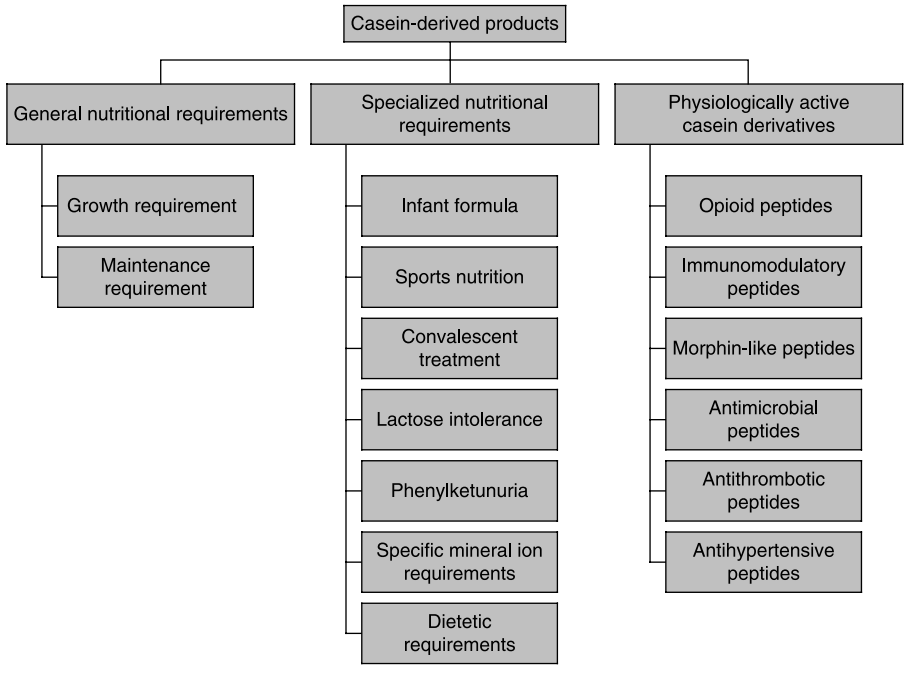

Figure 73.16 *Nutritional applications of casein-derived ingredients.*

wheat (PDCAAS = 0.40), or beans (PDCAAS ~ 0.60). This is due to the high digestibility of caseins and the high levels of essential amino acids. The nutritive value of a particular protein must also be considered in context of its value as part of a diet. For example, the lysine content of caseins is high while its sulfur-containing amino acid content is relatively low. However, when caseins are consumed in a diet containing cereal proteins (low lysine content but high in sulfur-containing amino acids), the amino acid requirements are easily

TABLE 73.6 Daily Amino Acid Requirements (mg/kg^{-1}/day) of Men, Women, Children, and Infants.

	Daily Amino Acid Requirement, mg Amino Acid/kg Body Weight				
Amino Acid	Women	Men	Children (10–12 years)	Children (2 years)	Infant (2–6 months)
His	?	?	?	?	34
Ile	9.5	10.1	28	31	119
Leu	12.5	15.7	42	73	150
Lys	9.4	11.4	44	64	103
Met	12.1	15.7	22	–	45
Cys	–	–	–	–	80
TSAA	12.1	14.4	22	27	125
Phe	12.1	15.7	22	–	90
Tyr	–	–	–	–	Present
TAAA	12.1	15.7	22	69	141
Thr	6.5	7.1	28	37	87
Trp	2.9	3.6	3.3	14	22
Val	10.7	11.4	25	43	105

Source: Harper (1981).

TABLE 73.7 Amino Acid Content of Different Proteins.

	Casein	Chicken (Muscle)	Chicken (Egg)	Fish	Beef (Muscle)	Pork (Muscle)
Nitrogen (% as is)	15.3	3.2	2.0	3.2	3.0	3.0
Arginine	239	348	381	354	410	380
Histidine	186	164	152	221	220	220
Isoleucine	345	334	393	299	320	320
Leucine	607	460	551	480	510	460
Lysine	518	497	436	569	540	510
Methionine	178	157	210	179	160	10
Phenylalanine	334	250	358	245	260	240
Threonine	297	248	320	286	280	290
Tryptophan	103	64	93	70	80	80
Valine	430	318	428	382	350	320
Cystine	23	82	152	73	80	80
Tyrosine	371	209	260	229	210	220
Alanine	196	213	370	374	360	290
Aspartic acid	455	573	601	647	580	590
Glutamic acid	1406	938	796	882	940	960
Glycine	126	331	207	301	390	310
Proline	738	259	260	230	310	280
Serine	385	244	478	271	260	260
Total amino acids	6940	5690	6450	6090	6260	5970
Total essential amino acids	3210	2620	3200	2810	2790	2690
Amino acid nitrogen	862	783	869	851	881	834
Amide nitrogen	98	58	79	58	52	62
Other nitrogen	20	159	52	91	66	104
N–P factor	5.99	489	5.54	5.23	5.37	5.13
AA score	91	99	100	100	100	100
Essential amino acid index	99	100	100	100	100	100
Protein efficiency ratio (PER)	2.9	–	3.8	–	2.9	–

Source: Bodwell (1981); Renner (1983).

met. A protein intake consisting of 76% milk protein and 24% cereal protein is deemed ideal in terms of protein requirements. Similarly when milk proteins and egg proteins are combined in a diet, the composite dietary value of the combination exceeds that of either protein individually (Fig. 73.17).

73.5.2 Specialized Dietary Requirements

73.5.2.1 Infant Nutrition. Milk is intended to be the exclusive source of nutrition to the neonate during a delicate period which combines pronounced growth, development of critical tissue (i.e., liver, kidney, etc.) and reduced tolerance due to the immaturity of the aforementioned tissue. The use of bovine milk in infant formula manufacture is widespread. However, bovine and human milk differ considerably (Table 73.9). Unmodifed bovine milk is not recommended for at least the first 6 months post partum and as a consequence slight adjustments are required (Poskitt, 1994; Table 73.8). The protein fraction of human milk supplies 6–8% of the available energy from milk which is lower than bovine milk (Morrow 1994; Poskitt 2004). The protein : carbohydrate : fat ratio similar to human milk (1 : 2 : 4) is conventionally formulated. The protein content of commercial infant food formulations is in the region of 1.3–4.0 g/100 mL. The casein : whey fractions

TABLE 73.8 Nitrogen-Protein Conversion Factors for Selected Proteins.

Protein	Nitrogen Conversion Factor
Casein	6.34
Wheat (wholegrain)	5.83
Maize	6.25
Rice	5.95
Beef	6.25
Egg	6.25
Soybean	5.71
β-Lactoglobulin	6.38

Source: Hambraeus (1992) and Grappin and Ribadeau-Dumas (1992).

of human and bovine milk are markedly different at 40 : 60 and 80 : 20, respectively. As a consequence the ratio is conventionally adjusted through the addition of whey protein concentrate. The net protein utilization (NPU) of infant food formulation is lower than that of human milk but higher then infant food formula manufactured using soy protein.

73.5.2.2 Dietary Requirements of Convalescent Patients. The importance of meeting the nutritional requirements of convalescent patients before and after operations can not be over emphasised. Enteral feeding procedures are an ideal way of meeting this requirement. As a consequence the development of high quality, easily digestible, casein derived products has received a lot of research attention. Micellar casein and casein hydrolysates with very specific free amino acid concentration and molecular weight profiles have been developed and are commercially available. The specific advantage of casein-derived material is that it is easily digestible and that the viscosity of such systems can be easily controlled. Milk protein preparations are used for patients suffering from gall

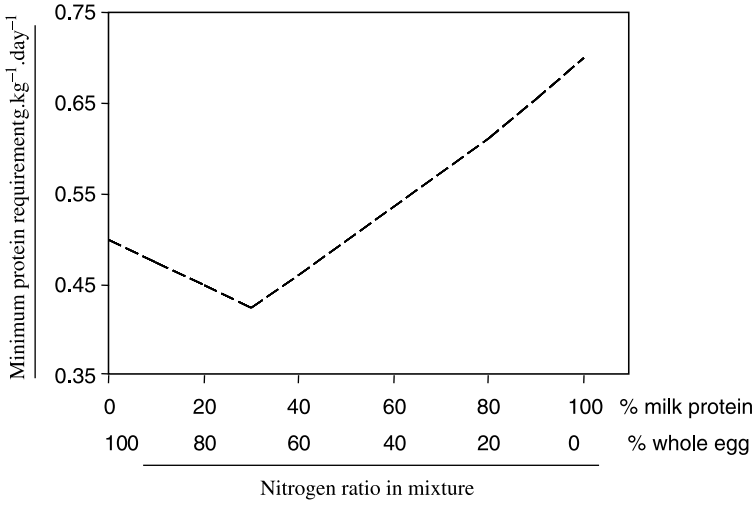

Figure 73.17 *Effect of protein intake on the minimum protein requirement (data from authors).*

TABLE 73.9 Comparison of the Composition of Bovine, Human, and Infant Formulae.

Constituent	Human Milk	Bovine Milk	Infant Formula	Follow-on-Formula
Energy, J/L	285.6	273.0	272.0	280.0
Total protein, g/L	9	30	15	25
Casein, % total protein	40	80	++	+++
Whey, % total protein	60	20	+++	++
β-Lactoglobulin, % total whey	ND	50		
α-Lactalbumin, % total whey	60	25		
Lactoferrin, % total whey	30	Trace		
Secretory IgA, % total whey	15	Trace		
Nonprotein nitrogen, ppm	250	50		
Urea	18	15		
Free amino acids	50	48		
Taurine	21.5	9.6		
Lactose, g/L	70	50	70	80
Fat, g/L	42	38	38	28
Poly/monounsaturated, g/kg total free fatty acids	500	250		
Ash, ppm				
Sodium	21	20	180	325
Chloride	17	30	350	700
Caclium	8	30	500	1000
Phosphorus	5	30	300	750

Source: Poskitt (1994); Morrow (2004).

bladder diseases, hyperlipidaemia, diabetes, cirrhosis, impaired kidney functions, gastric ulcers, and so on.

Micellar casein and casein hydrolysates are also widely used as nutritional supplements in protein based, powdered beverage products. The specific coagulation properties of caseins in the stomach also appears to aid digestion. It is unclear whether this effect is related to the micellar properties of caseins or the molecular properties in all casein monomers, that is, precipitation at isoelectric point (Renner 1983; Hambraeus 1992).

73.5.2.3 Lactose Intolerance. Lactose intolerance can be a congenital or secondary condition but in the majority of occasions is developmental, that is, genetically programmed to develop after about 2 years. Lactose intolerance is very common amongst some ethnic or racial groups, that is, incidence in black Africans, black Americans, and Asians is in the order of 75, 90, and 95%, respectively, and relatively low amongst other groups, that is, Caucasians (ca. 10%). Lactose intolerance is caused by the lack of β-galactosidase which is responsible for the breakdown of lactose in dairy products to glucose and galactose. In the case of individuals suffering from lactose intolerance, the unmetabolized lactose moves from the small intestine into the colon where its is catabolized by the endogenous gas-producing colon microflora. Symptoms include abdominal pain, flatulence, cramps, and to a lesser degree, abdominal distension and nausea. Symptoms usually manifest 30–120 min after the consumption of lactose. Common dietary adjustments adopted are to take β-galactosidase supplements or to avoid dairy products. The problem with the latter option is that dairy products are also a major dietary source of calcium and phosphate. The use of micellar casein or milk protein isolate to meet this requirement has been advocated. Caseinates or coprecipitates do not meet this

mineral supply requirement because they do not contain the endogenous colloidal calcium phosphate of native casein micelles. Supplementation of caseinate or precipitate preparations with calcium phosphate is not seen to be of the same nutritional value as micellar caseins as this source does not match the mineral profile of the minor mineral constituents.

73.5.2.4 Galactosaemia. Galactosaemia is a rare genetic condition resulting in the deficiency of one of three galactose metabolising enzymes (gal-1-P uridyltransfease, galactokinase, or UDP galaose-4-epimerase). When lactose is consumed it is metabolized to glucose and galactose which results in a build up of galactose in the brain and eyes causing severe mental retardation and eye cataracts. The replacement of standard skimmed milk protein with micellar milk protein would eliminate any potential problems associated with galactosaemia.

73.5.2.5 Phenylketonuria. Phenylketonuria (PKU) is a autsomal recessive condition where there is little if any ability to metabolize phenlyalanine due to a near complete deficiency of phenylalanine hydroxylase. High phenylalanine and tyrosine levels in blood may cause brain damage such as profound and irreversible mental retardation, microcephaly, or epilepsy. Unless phenylalanine levels in food are tightly controlled, especially during childhood, some mental impairment is inevitable. Standard food preparations for individuals suffering from PKU include amino acid mixtures and synthetic peptide systems. The use of the κ-casein glycomacropeptide as stable source of amino acids for individuals suffering from PKU has also been advocated (Renner 1983; Nielsen and Tromholt 1994; Brody 2002). The lack of essential amino acids such as cysteine, histidine, tryptophan, tyrosine necessitates that some other source of amino acids be consumed in addition to GMP.

73.5.2.6 Hyperuricaemia and Related Conditions. It would appear that milk proteins are an ideal source of protein for individuals suffering from hyperuricaemia and gout. Both conditions are caused by the build up and subsequent deposition of uric acid in the muscle and tissue. Dietary purines are the principal precursors for uric acid. Casein-derived products are a very suitable source of purine low protein (Renner 1983).

73.5.2.7 Sports Nutrition. The dietary requirements of athletes are very specific. It is required that sufficient protein is consumed to provide amino acids for synthesis of body protein during growth and replace body proteins irreversibly catabolized during exercise. Physical exercise regardless of its specific nature (high intensity/short time vs. endurance exercise) has a similar acute response (decrease in protein synthesis and increased rate of protein degradation) but the time course and magnitude of the changes differ. Endurance exercise causes an increase in mitochondrial protein with minimal effect on the myofilbrillar proteins, while weight lifting causes an increase in catabolic response and decrease in anabolic response. It has been proposed that the recommended daily protein intake of athletes is approximately 1.3–1.6 g/kg/day (Gontzea and others 1974, 1975; Friedman and Lemon 1989). Caseinates, coprecipitates and casein hydrolysates are commonly used with whey protein (whey protein isolate or whey protein concentrate) in protein supplement beverage powder mixes.

It has also been proposed that "slow release proteins" such as micellar casein could be used by athletes to prevent muscle catabolism during periods of dieting or prolonged fasting. The basis of the "slow release proteins" claim is that casein coagulates and

aggregrate in the stomach causing slow degradation of proteins with a concomitant slow release of amino acids. This in turn decreases the rate of muscle degradation (Boirie and others 1997; Beanfrere and others 2000; Manninen 2002).

73.5.2.8 Weight Reduction Diets. Casein and casein-derived products have two specific applications within weight reduction diets. Pure casein products, particularly micellar casein preparations, could be viewed as carbohydrate-free variants of skimmed milk powder. The benefit of micellar casein over coprecipitates/caseinates is that the former system is rich in calcium, phosphorus and other minerals and as such mimics skimmed milk more closely than coprecipitates or caseinates. The applications of micellar casein as an alternative to skimmed milk solids include low carbohydrate ice cream, low carbohydrate yogurt, dairy smoothies, and so on.

The κ-casein glycomacropeptide has been demonstrated to increase the sensation of satiety and induces an appetite-reducing effect. Satiety is loosely defined as the feeling of fullness. It has been proposed that ingestion of κ-casein glycomacropeptide causes a increase in the release of cholecystokinin (CKK) in the duodenum and jejunum with a concomitant increase in CKK concentration in the blood. CKK stimulates the production of insulin and slows the rate of gastric emptying which in turn contributes to a long lasting sensation of fullness. It would appear that the sialic acid and sugar moieties of GMP play a paramount role in this physiological effect (Beucher and others 1994; Corring and others 1997; Monnai and Otani 1997; Froetschel and others 2001).

73.5.3 Biologically Active Peptides

73.5.3.1 Opioid Peptides. Casein-derived biologically active peptides have received a lot of research attention recently and this trend is likely to continue. Casein-derived bioactive peptides have a range of physiological effects (Fig. 73.16 and Table 73.10). Opioid-like peptide are the most studied biologically active casein peptides and can be subdivided into opioid agonists and opioid antagonists. Opioid agonists are prepared from α_s-/β-casein hydrolysis and act by binding to naloxone receptors (Kilara and Panyam 2003). The proposed physiological effects of casein opioid agonists are modulation of social behavior, lengthening gastrointestinal transit duration, antidiarrheal action, stimulating secretion of insulin, and somatostatin and simulating intestinal transport (Takahashi and others 1997; Meisel and FitzGerald 2000; Floris and Alting 2003). Casein antagonists suppress the action of humoral peptides enkephalins and block the action of agonists. It should be noted that the specific effect of casein-derived opioid peptides may depend on the mode of administration (parenteral feeding vs. oral administration). A lot of research is required to elucidate the actual physiological effect of ingesting biologically active peptides.

73.5.3.2 Immunoregulatory Peptides. Several casein derived peptides have been shown to have defined imunoregulatory properties. Casein-derived peptides may directly inhibit the growth of bacteria (*Encherichia coli*, *Straphyococcus aueus*, or *Klebsiella pneumoniae*), yeasts (*Candidia albicans*), or viruses (Epstein Barr virus), or alternatively enhance the existing immune system by increasing antigen T cell proliferation, lymphocyte proliferation, neutrophil locomotion, phagocytosis, peritoneal macrophage activity, murine activity, or natural killer cell activity (Coste and others 1992; Lehov and Regelson 1996; Otani and Monnai 1996; Otani and others 1996; Brody 2000) FitzGerald and Meisel

TABLE 73.10 List of Selected Biologically Active Peptides Derived from Casein.

Role	Peptide	Comment	Ref.
Mineral binding	α_{s1}-CN F43–59	Binds Fe, Mn, Cu, Se, Ca,	[a]
	α_{s1}-CN F43–58	Binds Fe, Ca; two phosphoserine residues, net charge of −7	[a]
	α_{s1}-CN F43–79	7 Phosphoserine residues, binds Ca	[a]
	α_{s1}-CN F59–64	2 Phosphoserine residues, binds Ca	[a]
	α_{s1}-CN F59–79	Binds Ca and Fe; 5 phosphoserine residues, net charge of −9	[a]
	α_{s2}-CN F1–21	4 Phosphoserine residues	[a]
	α_{s2}-CN F46–70	4 Phosphoserine residues; charge of −11	[a]
	α_{s2}-CN F66–74	3 Phosphoserine residues; binds Ca	[a]
	β-CN F1–25	Binds Ca and Fe; 4 phosphoserine residues, net charge of −9	[a]
	β-CN F1–28	Binds Ca; 4 phosphoserine residues; charge of −8	[a]
	β-CN F33–48	Binds Ca; charge of −6	[a]
Antithrombotic	κ-CN F106–116	Inhibits thrombin-induced aggregation and ADP-induced platelet aggregation	[b]
	κ-CN F106–112	ADP-induced platelet aggregation	[b]
	κ-CN F113–116	ADP-induced platelet aggregation	[b]
	κ-CN F103–111	Inhibits thrombin-induced aggregation and ADP-induced platelet aggregation	[b]
	κ-CN F112–116	Inhibits thrombin-induced aggregation and ADP-induced platelet aggregation	[b]
Immunoregulatory peptides	α_{s1}-CN F194–199	Increases antibody formation, increases phagocytosis, decreases proliferation of lymphocytes, ACE inhibitor	
	α_{s1}-CN F1–23	*Isracidin*; Protection against *Straph. aureus*, *E. coli*, and *Candidia albicans*	[c]
	α_{s1}-CN F90–96	Decreases lymphocyte proliferation, increases natural killer activity and increases neutrophil locomotion	[c]
	α_{s1}-CN F90–95	Decreases lymphocyte proliferation, increases natural killer activity and increases neutrophil locomotion	[c]
	α_{s1}-CN F23–34	ACE inhibtor, increases phagotosis, protects against *Klebsiella pneumoniae* infection	[c]
	α_{s1}-CN F23–37	ACE inhibtor	[f]
	α_{s1}-CN F28–34	ACE inhibtor	[f]
	α_{s1}-CN F104–109	ACE inhibtor	[f]
	α_{s1}-CN F142–147	ACE inhibtor	[f]

(*Continued*)

TABLE 73.10 *Continued.*

Role	Peptide	Comment	Ref.
	α_{s1}-CN F143–148	ACE inhibtor	*f*
	α_{s1}-CN F157–164	ACE inhibtor	*f*
	α_{s1}-CN F194–199	ACE inhibtor	*f*
	β-CN F193–209	Decreases lymphocyte proliferation, increases phagocytosis	*c*
	β-CN F63–68	Increases antibody formation, increases phagocytosis	*c*
	β-CN F191–193	Increases antigen dependent T cell proliferation	*c*
	β-CN F60–70	Decreases lymphocyte proliferation; protects against *Klebsiella pneumoniae* infection	*c*
	β-CN F60–66		*c*
	β-CN F74–76	ACE inhibitor	*f*
	β-CN F57–64	ACE inhibitor	*f*
	β-CN F84–86	ACE inhibitor	*f*
	β-CN F108–113	ACE inhibitor	*f*
	β-CN F169–175	ACE inhibitor	*f*
	β-CN F177–183	ACE inhibitor	*f*
	β-CN F193–198	ACE inhibitor	*f*
	β-CN F193–202	ACE inhibitor	*c*
	β-CN F77–183	ACE inhibitor	*c*
	κ-CN F106–169	*Glycomacropeptide*; inhibits splenocyte proliferation	*g*
	κ-CN F106–169	*Glycomacropeptide*; binds cholera toxin	*g*
	κ-CN F106–169	*Glycomacropeptide*; binds *E. coli* heat labile enterotoxins LT-I and LT-II	*g*
	κ-CN F106–169	*Glycomacropeptide*; adhesion and protection against Epstein-Barr virus	*g*
	κ-CN F106–169	*Glycomacropeptide*; inhibition of cariogenic bacteria such as *Streptococcus mutans*, *S. saguis*, *S. sobrinus and Actinomyces viscosus*	*h*
	κ-CN F106–169	*Glycomacropeptide*; Decreases lymphocyte proliferation	*c*
	κ-CN F17–21	Decreases lymphocyte proliferation	*c*
	κ-CN F38–39	Decreases lymphocyte proliferation	*c*
	κ-CN F58–59	ACE inhibitor	*f*
	κ-CN F108–110	ACE inhibitor	*f*
Opioid	β-CN F 60-64	β-*Casomorphin 5*; Opioid agonist	
	β-CN F	β-*Casomorphin 5*; Opioid agonist	*g*
	β-CN F	*Morphiceptin*; Opioid agonist	*g*
	α_{s1}-CN F	α-*Casein exorphin*; Opioid agonist	*g*
	α_{s1}-CN F	*Casoxin D*; Opioid antagonist	*g*
	κ-CN F	*Casoxin 4*; Opioid antagonist	*g*
	κ-CN F35–42	*Casoxin A*; Opioid antagonist	*h*
	κ-CN F58–61	*Casoxin B*; Opioid antagonist	*h*

(*Continued*)

TABLE 73.10 *Continued.*

Role	Peptide	Comment	Ref.
	κ-CN F25–34	*Casoxin C*; Opioid antagonist	[h]
DNA synthesis stimulating	β-CN F177–183		[f]
	β-CN F105–69		[f]
	κ-CN F106–169	*Glycomacropetide*	[f]
Antithrombotic activity	κ-CN F106–116	Inhibits ADP-treated platlet aggregation	[f]
	κ-CN F39–42		[f]
	κ-CN F103–116		[f]
	κ-CN F106–169	*Glycomacropetide*, Antithrombotic activity	[f]
	κ-CN F106–110	*Casopiastrin*	[f]
Suppress gastric secretions	κ-CN F106–169	Acidity of gastric secretions lowered and motions in gastric fundus and duodenum reduced.	[g]
Bfidobateria stimulating	κ-CN F106–169	*Glycomacropeptide*; only glycosylated form is active	[g]
Antitumor	Casein	Prevention of DHM-induced intestinal cancer	[i]

[a]Vegarud and others 2000; [b]Rutherfurd and Gill 2000; [c]Gill and Cross 2000; [d]Shah 2000; [e]Meisel and FitzGerald 2000; [f]FitGerald and Meisel 2000; [g]Brody 2000; [h]Nesser and others 1988; [i]Gill and Cross 2000.

2000; Gill and Cross 2000; Harsharnjit and others 2000; Floris and Alting 2003; Kilara and Panyam 2003). The specific immunoregulaory activity of casein hydrolysates are presumably due to the distinct surface properties of casein peptides. It has also been demonstrated that κ-casein promotes the growth of Bifidobacteria. It would appear that glycosylated forms of GMP are the active variants (Zbikowski and Ziajkas 1986; Yvon and others 1994; Brody 2000).

The use of GMP as an anticariogenic agent for the protection of teeth has also been proposed. It has been reported that GMP inhibits the adhesion of cariogenic bacteria such as *Streptococci mutans*, *S. sorbinus*, *Actinomyces visosus*, to teeth (Schupbach and others 1996; Vacca Smith and others 2000).

73.5.3.3 Antihypertensive Peptides. The ability of casein-derived peptides to inhibit angiotensin-I-converting enzyme has received a lot of research attention. Angiotensin-I-converting enzyme (ACE), a dipeptide liberating carboxypeptidase (peptidydipepide hydrolase; EC 3.4.15.1) converts angiotensin I to octapeptide angiotensin II and also hydrolyses brandykinin (FitzGerald and Meisel 2000; Grosiak and Muller 2000; Shah 2000). Angiotensin I is a potent vasoconstrictor. Casein-derived ACE inhibitors (casokinins) have charged amino acid moieties at their C-terminal and are thought to inhibit ACE by electrostatically binding to the catalytic site of ACE (see FitzGerald and Meisel 2000). A specific feature of casokinins is their resistance to digestive hydrolysis which may be related to their relatively high proline content.

73.5.3.4 Mineral Binding Peptides. The ability of casein to bind and enhance the delivery of minerals is the intrinsic biological role of casein (Holt 1992a). It has also been demonstrated that casein-derived peptides can bind and enhance the adsorption of mineral ions (Scholz and Schrezenmeir 2000; Vegarud and others 2000; Floris and Alting 2003). The binding mechanism of casein can be specific through phosphserine residues or nonspecific. The use of casein derived peptides as mineral binding agents in dental care products (to counteract decalcification of dental enamel), breakfast cereal products and dry flour mixes has also been advocated (Reynolds 1987).

73.5.3.5 Antithrombotic Peptides. Several κ-casein derived peptides have been demonstrated to have antithombotic properties. This effect is presumably related to the high degree of homology between the human fibrinogen γ-chain and κ-casein peptide fraction F106–116. Collectively antithrombotic κ-casein derived peptides are called casoplatelins and are thought to work by (1) physically inhibiting platlet aggregation, and (2) inhibiting the fibrinogen γ-chain binding to the platlet surface (Jolles and others 1986; Fossett and Tome 1998; Tome and Ledoux 1998; Pfeuffer and Schrezenmeir 2000; Rutherford and Gill 2000; Floris and Alting 2003; Kilara and Panyam 2003).

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74

Ice Cream and Frozen Desserts

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74.1 Introduction 594
74.1.1 Classification of Frozen Desserts 595
74.1.2 In the United States, FDA Standards 595
74.1.3 Trade Classification 596
74.1.4 Trends in Ice Cream Market 596
74.2 Formulation 597
74.2.1 Concentrated Sources of Milk Fat 599
74.2.1.1 Sweet Fresh Cream and Fresh Milk 600
74.2.1.2 Frozen Cream 600
74.2.2 Concentrated Sources of Serum Solids 601
74.2.2.1 Fluid Whole and Skim Milk 601
74.2.2.2 Plain Condensed Skim Milk 601
74.2.2.3 Plain Condensed Whole Milk 601
74.2.2.4 Superheated Condensed Skim or Whole Milk 602
74.2.2.5 Sweetened Condensed Whole or Skim Milk 602
74.2.3 Balancing Ingredients 602
74.2.4 Sweeteners 602
74.2.4.1 Nutritive Sweeteners 602
74.2.4.2 Nonnutritive Sweeteners 604
74.2.5 Stabilizers and Emulsifiers 604
74.2.5.1 Stabilizers 605
74.2.5.2 Emulsifiers 606

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

74.3 Mix Calculations 606
74.3.1 Terms Used in Mix Calculations 607
74.3.2 Arithmetic Method 609
74.3.2.1 Serum Point Formula 610
74.3.2.2 Milk and Cream Formula 610
74.3.3 Mix Proof Sheet 611
74.4 Processing 613
74.4.1 Blending 613
74.4.2 Pasteurization 615
74.4.3 Homogenization 616
74.4.4 Aging 617
74.4.5 Flavors 617
74.4.6 Freezing and Hardening 620
74.4.6.1 Refrigeration 620
74.4.6.2 Freezing Ice Cream 621
74.4.6.3 Overrun 621
74.4.6.4 Types of Ice Cream Freezers 622
74.4.6.5 Hardening 624
74.5 Packaging 624
74.5.1 Storage and Distribution 626
74.5.1.1 Manufacturing Procedures 626
74.5.1.2 Warehouse Equipment 627
74.5.1.3 Transportation 627
74.5.1.4 Storage on Retail Premises 627
74.5.1.5 Retail Display Equipment 627
74.5.1.6 Retail Handling Practices 628
74.5.1.7 Heat Shock 628
74.6 Nutritional Value of Ice Cream 630
74.7 Manufacture of Ice Cream Novelties 630
74.7.1 Molded Novelties 630
74.7.2 Extruded Novelties 632
Further Reading 633

74.1 INTRODUCTION

Despite of the myths surrounding the origins of ice cream, we do not know for sure how the evolution of ice cream resulted. Snow and ice have been popular during warmer seasons because of the cooling properties of these materials. Perhaps first snow and ice were mixed with fruit juices and later with milk or yogurt, and this resulted in a gradual evolution of these products as we know them. Until the nineteenth century, harvesting ice and storing it for use during summer was a labor intensive process and therefore, ice cream was a food for the rich only. With the invention of the hand cranked freezer and a ready availability of ice, ice cream moved down the social ladder, and towards the end of the nineteenth century it was sold on the streets of metropolitan areas.

Ice cream and frozen desserts are popular throughout the world. Although ice cream is a popular frozen dessert in all parts of the world, the major consumer countries of ice cream are New Zealand, the United States, Canada, Australia, Belgium, Finland, and Sweden. In some areas of the world, unavailability of appropriate ingredients, lack of refrigerated distribution chain, economics, or other cultural factors may deter the manufacture (and

therefore consumption) of ice cream. There are several different names for ice cream in the world. In Norwegian it is *iskrem*, in Portuguese *sorvettes*, in Spanish *healdos*, in French *glacé*, in Italian *gelato*, in Hebrew *glidah*, in German *eis*, in Finnish *jatelo*, in Greek *pagoto*, and in Chinese *bing qi lin* or *sou go*.

The popularity of ice cream results from several characteristics such as partial freezing, cooling, and refreshing sensation produced when the product is consumed, its sweet taste and the lack of a preconditioning aroma. In all these products a sugar solution is the common denominator. The characteristics of the syrup are manipulated by the addition of other materials to obtain desired taste, texture, consistency, and appearance.

Ice cream is manufactured as regular, custard/French, reduced fat, light, low, and no-fat versions. Other frozen desserts include frozen yogurt, sherbet, water ice, mellorine, frozen dairy dessert, frozen confection, frozen dairy confection, milk shake, smoothies, shake, and slush. The nomenclature varies from country to country depending on the prevailing legislation. Two manufacturing practices that affect the characteristics of frozen desserts are the freezing technique and degree of freezing. The freezing technique may involve stirring (agitation) during freezing, or without stirring (quiescent), or a combination of the two. Similarly, the degree of freezing results in products that are hard frozen, or designed for dipping or scooping, or used as soft serve or a milkshake.

The characterizing materials that are added to the sugar syrup are dairy ingredients, sweeteners (other than sucrose), body and texture modifiers, flavors, and colors. Most of the legal terms used in this chapter are based on the authors' experience in the United States and it is recognized that this information varies considerably due to geography.

74.1.1 Classification of Frozen Desserts

74.1.2 In the United States, FDA Standards

The Code of Federal Regulations (21 CFR135.110) specifies both compositional and manufacturing requirements for a product to be called ice cream. The compositional requirements state that the product cannot contain less than 20% total milk solids including 10% milk fat. No more than 25% of the nonfat milk solids can be derived from whey, and caseinates may be used only after reaching the 20% milk solids minimum. Some allowances are made for bulky flavors such as chocolate and certain fruit flavors, and the requirements for milk solids level is reduced by 20%. For a product to be called frozen custard (or French ice cream), the product must contain at least 1.4% egg yolk solids. Last but not the least, a minimum total solids and weight per gallon are also specified. Ice cream must weigh a minimum of 4.5 lb/gallon or 540 g/L. This requirement specifies the maximum amount of air (a maximum of 100% overrun) permissible in the product. However, for reduced fat, low fat and nonfat ice cream, the minimum weight requirement is reduced to 480 g/L. Total food solids must be at least 1.6 lb/gallon or 192 g/L. A serving of ice cream is considered to be one half cup or 4 fl. oz, or 120 mL (a volumetric measure). To conform to the weight requirement of 4.5 lbs/gallon, 1/2 cup corresponds to 63.8 g.

The manufacturing requirements defined in law are that the freezing should occur under agitation; the mix should be pasteurized, may contain safe and suitable nonmilk derived ingredients, may be sweetened with safe and suitable sweeteners, and may be flavored.

With the introduction of the Nutritional Education and Labeling Act (NLEA) in 1993 several modifying terms could be used with standardized foods. Thus descriptors such as reduced fat, light, low fat and nonfat, or no fat could be used in conjunction with the word ice cream. Reduced fat ice cream is a nutritionally altered product (compared to ice cream)

which contains 25% less fat as compared to conventional ice cream. Light or Lite ice cream is a product that has one-third fewer calories or half the amount of fat of that found in regular ice cream. Low fat ice cream is a product that contains less than 3 g fat per serving. Fat free, no fat, or nonfat ice cream is a product that contains less than 0.5 g fat per serving. With these changes in descriptors, the total milk solids requirements of 20% still apply and all other aspects of ice cream described above still apply.

There are other frozen desserts that also have a standard of identity. These are sherbet, mellorine, and water ice. Sherbet should contain not less than 1% milk fat and no more than 2% milk fat; milk solids-not-fat content should be not less than 2% and no more than 5%. The minimum weight requirement is 6 lb/per gallon. A fruit flavored sherbet should have a minimum acidity of 0.35%. Sorbet and water ices have the same standards as sherbet except no milk or egg ingredient is allowed (except egg white). Frozen yogurt are has no federal standard of identity, but it consists of a mixture of dairy ingredients, sweeteners, stabilizers, and flavors. Usually, frozen yogurt mix contains 10% cultured yogurt component and the titratable acidity of the mix is 0.3%. Novelties are packaged in single servings. They may be juice bars, fudge sticks, or ice cream sandwiches. Gelato is served in a semifrozen state resembling soft serve ice cream. It is more dense (contains less air) than ice cream.

Mellorine is a product made with fats other than milkfat. These fats can be animal or vegetable derived. Mellorine should contain a minimum of 1.6 lb of food solids per gallon, a fat content of not less than 6%, a minimum protein content of 2.7% (the protein has to be of equal nutritional value of milk protein) and a gallon of mellorine should weigh not less than 4.5 lb per gallon.

When a pasteurized ice cream mix is frozen without stirring the product is labeled as a quiescently frozen dairy dessert. If the product contains no milk solids or less than a total of 20% total milk solids and is frozen without agitation it has to be labeled as quiescently frozen dairy confection. On the other hand, if the product does not contain any dairy ingredients and is frozen without agitation then it is called a frozen confection.

74.1.3 Trade Classification

The chemical composition of ice cream differs mainly with regard to the fat content and three grades of ice cream can usually be found in most market areas. One grade just meets the minimum fat content, often has an overrun that approaches the maximum allowed by law, and usually contains relatively inexpensive flavor ingredients. At the other extreme are the so-called premium ice creams that are high in fat, low in overrun, and usually contain natural flavors. A third grade of ice cream, designed as a compromise between the minimum cost and premium products, is the type that has dominated the market for many years. Newer developments have introduced a fourth grade referred to as super premium ice cream that is characterized by higher fat contents and lower overruns than premium varieties. Cost of the product is directly proportional to the grade of the ice cream.

74.1.4 Trends in Ice Cream Market

The volumes of ice cream and other frozen dairy desserts produced in the United States in 2003 and 2004 are shown in Table 74.1.

According to U.S. Department of Agriculture statistics, 1.58 billion gallons of ice cream and frozen desserts were manufactured in 2004. Of this total, 60% was ice

TABLE 74.1 Production Statistics of Various Frozen Desserts in the United States.

Frozen Dessert	2003 (Million Gallons)	2004 (Million Gallons)
Ice cream, regular, total	992.9	943.7
Ice cream, regular, hard variety	875.4	844.7
Ice cream, low fat, total	398.3	415.5
Ice cream, low fat, hard variety	101.4	114.1
Ice cream, low fat, soft variety	296.9	301.4
Ice cream, nonfat, total	20.4	22.7
Ice cream, nonfat, hard	18.8	19.1
Water juices and ices	60.6	63.9
Frozen yogurt, total	70.4	67.7
Frozen yogurt, low fat, hard	21.4	19.7
Frozen yogurt, nonfat, hard	12.1	11.1
Other frozen dairy desserts	7.2	8.1

Source: http//www.usda.gov/mass/pubs/agro5/05-ch8.PDF.

cream, 28% was reduced fat, low- fat and nonfat ice cream, 4% frozen yogurt, 4% water and juices, 3.5% sherbet, and 0.5% other frozen products. It is estimated that 444.2 million gallons of soft serve ice cream products were produced in 2004. In the United States, per capita sales in 2004 were 26.4 quarts and the market value exceeded 21 billion dollars.

74.2 FORMULATION

In order to make an ice cream mix, three categories of ingredients are necessary. A concentrated source of milk fat is the first category, the second is a concentrated source of milk solids-not-fat (aka serum solids) and the third is called a balancing ingredient.

Representative formulae for commercial grades of ice ream are shown in Table 74.2. Composition of other variants of ice cream and frozen desserts are shown in Table 74.3. The prioritization of ingredient selection can be said to approximate the hierarchy as follows:

1. *Select Milk Fat Content*: Since milk is bought and sold on the basis of fat content and quality grade of ice cream is also reflected by the fat content, the amount of milk fat will determine the cost and grade of the product.

TABLE 74.2 Representative Formulae for Ice Creams of Different Grades.

			Super Premium %		
Constituent	FDA Standard %	Premium %	1	2	Super %
Milk fat, min.	10.00	12.00	14.00	16.00	18.00
Milk solids–not-fat, min.	7.50	9.00	10.00	10.50	9.50
Whey solids, max.	2.50	2.00	–	–	–
Sucrose	–	7.60	12.00	15.00	15.00
Corn syrup solids	–	9.00	6.80	5.00	–
High fructose solids	–	4.50	2.60	–	–
Stabilizer	–	0.35	0.25	0.13	0.12
Emulsifier	–	0.25	0.25	0.15	0.10
Total solids	–	40.50	41.28	41.72	42.50

TABLE 74.3 Composition of Other Frozen Desserts.

Constituent	Ice Cream			Sherbet	Water Ice
	Nonfat	Low Fat	Reduced Fat		
		Abundance (%)			
Milk fat	0.5	3.0	6.0	1.5	0
MSNF	13.5	13.0	12.5	3.5	0
Sucrose	10.0	9.0	10.0	23.0	23.0
Corn syrup solids	10.0	9.0	8.0	7.0	7.0
Stabilizer/emulsifier	1.0	0.8	0.6	0.4	0.4
Total solids	35.0	34.8	37.1	35.4	30.4
Water	65.0	65.2	62.9	64.6	69.6
Total solids	35.0	34.8	37.1	35.4	30.4

2. *Select Nonfat Milk Solids Level* to complement the fat content. Too much of nonfat milk solids can cause sandiness.
3. *Sweetener Ingredient*: Type and amount of sweetener are in turn dependent on the fat and total solids content and also by economic considerations. Higher fat mixes need high sweetener levels. Type of sweetener used depends on demands and supply which in turn controls the cost of the ingredients.
4. *Stabilizer and Emulsifier*: The amount and type are affected by fat and total solids levels, manufacturing processes, and storage and distribution factors.
5. *Label Considerations*: If specific nutritional or other claims have to be made (e.g., "all natural," or "reduced calorie," or "reduced fat") then the above hierarchy of ingredient selection is slightly modified and cost of ingredients plays a lesser role than the functional aspects.

For the manufacture of good ice cream there is no secret or magic formula. Difference between good and not-so-good ice cream is controlled management. By controlled management is meant such things as using good ingredients for every batch, maintaining the same composition every time, proper and controlled mix making, freezing, packaging, hardening, storage, distribution, and minimal turnover time.

These representative subclasses vary not only in the proportion of ingredients but also in the types of ingredients used to furnish a required component. For example, in the "economy" subclass of ice cream serum solids may be derived from nonfat dry milk (skim milk powder), or condensed skim milk and can also include the maximum allowable amounts of whey solids. The maximum allowed amount of whey solids is 25% of the milk solids-not-fat content. Therefore the 10.0% of serum solids may be derived from condensed skim or nonfat dry milk up to a total of 7.5% and whey solids may be added to the level of 2.5%. Since whey solids cost considerably less than milk solids, thereby the overall costs of the formulation can be reduced. Similarly, sweeteners may also be substituted to reduce costs of formulations. High fructose corn syrup has approximately the same sweetness intensity as sugar but cots less than sugar. In some formulations, approximately 50% of the sugar solids can be substituted with high fructose corn syrup solids. In general, the "economy," "regular," and "premium" subclasses of ice cream also contain higher overrun while the "super premium" subclass contains less overrun. Additionally, the "super premium" subclasses are packaged in smaller containers of 468 mL (1 pint) whereas

the other products are packaged in 1.874 L (half U.S. gallon) containers. Lastly, the distribution and points of sales of the different subclasses of products can also vary considerably.

74.2.1 Concentrated Sources of Milk Fat

Fat in milk is secreted as tiny droplets called globules. A drop of milk contains millions of such globules. Each globule is surrounded by a membrane called milk fat globule membrane. This fat is made up of triglycerides, traces of di- and monoglycerides, cholesterol, and phospholipids among many other substances. In fact milk fat is composed of some 3600 different compounds. The triglycerides which are the main component are synthesized by the cow by linking three molecules of fatty acids to one molecule of glycerol, hence the name triglycerides. These fatty acids consist of carbon, hydrogen, and oxygen atoms. Fatty acids can have as few as four carbon atoms or as many as 26 carbon atoms. In milk fat, we have a number of different fatty acids including those containing four, six, and eight carbon atoms. This is significant because the characteristic flavor of milk fat is in large part due to the presence of these lower chain fatty acids butyric, caproic capryllic, and capric acids, carbon numbers 4, 6, 8, and 10, respectively. Further there are fatty acids that are unsaturated and thus we have fatty acids with one double bond (monoenoic) fatty acids, two double bonds (dienoic) or with three fatty acids (trienoic). The unsaturated fatty acids with multiple double bonds are functional, healthy, and essential for human beings.

Milk fat melts and crystallizes, is unctuous, depresses the cold sensation, contributes desirable flavor, is a solvent for added flavors, adds structure to ice cream and is of great importance in extrusion properties of ice cream. Extrusion helps shape ice cream in novelties.

Milk and cream constitute the most important components because they furnish the basic ingredients for a good quality ice cream. Variables related to dairy ingredients exert a profound influence on the flavor, body, and texture of the frozen product.

The nature and intensity of ice cream flavor is a function of the flavor quality of the individual constituents and subsequent processing treatment of the ice cream mix. Flavor defects in ingredients cannot be alleviated during ice cream making. Actually, flavor problems could be compounded as a consequence of negligent processing procedures.

The body or consistency of ice cream is related to the mechanical strength of the mix and its resistance to melting. Heat shock resistance is dependent on the nature and concentration of the stabilizer-emulsifier system used. The texture of ice cream depends upon the size, shape, number, and arrangement of fat globules, ice crystals, and the ratio of liquid and frozen water in the ice cream.

Balancing quality and cost is a major challenge to frozen dessert manufacturer. Satisfactory composition produces ice cream having an optimum combination of cost, flavor, body, and texture, cooling effect, viscosity, whipping ability, and freezing characteristics. In summary, factors responsible for overall ice cream quality are: raw material quality, sanitary care during mix preparation, processing parameters, flavoring used, freezing techniques, and storage conditions.

Formulation of frozen dessert mix involves utilization of both the fat and solids-not-fat components of milk. The functions and preferred sources of major ice cream ingredients are summarized in Table 74.4.

TABLE 74.4 Role and Sources of Various Components of Ice Cream.

	Role and Functions	Limitation	Sources in Order of Preference
Milk fat	Imparts desirable creamy rich flavor Source of fat-soluble vitamins Improves body texture Improves melting resistance	Calorie-dense. Too much fat (17%) gives too much viscosity to mix and hinders whipability Source of oxidized, rancid, and fishy flavor defects	Fresh sweet cream Fresh milk Frozen cream
Milk-solids-not-fat	Improves texture Imparts better body Source of protein, minerals, and vitamins	Improper levels cause "sandiness" defect Source of desirable cooked flavor	Dry milk Fluid whole milk Fluid skim milk Condensed skim milk Skim milk powder, whey products
Sweeteners	Impart sweet flavor	Too much sweetener, especially corn syrup impedes freezing process	Cane sugar, corn syrup solids, high fructose corn syrup

74.2.1.1 Sweet Fresh Cream and Fresh Milk. Whole milk may be used primarily as a source of milk solids. Without question, there is no better source of fat than sweet cream because of its desirable flavor, convenience of handling, and good whipping characteristics. Fresh cream is judged by flavor, acidity, and bacterial count. The titratable acidity should be low and show no evidence of developed acidity. When fresh cream is not available at a favorable cost, alternative sources of fat should be considered.

74.2.1.2 Frozen Cream. The high price of sweet cream during certain seasons of the year makes storage of cream during the months of surplus economically attractive. All known precautions must be used to ensure prevention of the development of off-flavors in stored cream. Only the best cream should be processed for storage, and it should contain no developed acidity. Off-flavors likely to develop in frozen cream are rancid, fishy, oily, and tallowy. Hydrolytic rancidity is due to free butyric acid from the partial hydrolysis of butter fat brought about by enzymatic activity of lipase on the butterfat or by certain bacteria of the enzymes they produce. A proper heat treatment regime, an essential phase for the preparation of cream for freezing, consists of heating cream at 76.7°C for 20 min, or 82.2°C for 10 min, or 87.8°C for 5 min. This treatment not only inactivates the lipase enzyme naturally present in milk but also destroys 95–99% of the bacteria present.

Heat treatment of cream also increases the resistance of the cream to oxidation. A fishy flavor in dairy products results from the formation of trimethylamines by the hydrolysis and oxidation of lecithin, naturally occurring phospholipids in milk. Factors that promote development of this flavor are high acidity and the presence of proxidants (iron or copper salts). Evidently, heat treatment at these times and temperatures "activates" or uncoils the proteins so that sulfhydryl groups are exposed and become oxidized by atmospheric oxygen in preference to the unsaturated fatty acids. Apparently, these sulfhydryl groups function as antioxidants in the liquid system. In addition they may complex with proxidant minerals.

Following heat processing, the cream is quickly frozen. Proper packaging and handling of frozen cream are also important. Preferred packages include stainless steel or plastic

containers. Quick-frozen cream is held at −20°C. Disadvantages of frozen cream include the necessity or thawing before use and the fact that it is messy to handle.

Due to fluctuating supplies and price of cream and the disadvantages of frozen cream some manufacturers rely on unsalted sweet cream butter. Butter is manufactured by agitating or churning cream. Cream is oil-in-water emulsion. The aqueous phase is skim milk. Agitation of cream results in a phase inversion converting oil-in-water emulsion to water-in-oil emulsion. The serum is separated and is called buttermilk. This buttermilk is not to be confused with cultured buttermilk which is a different product. Buttermilk is dried into a powder form and can be used as a source of serum solids in ice cream formulations. Unsalted butter is packaged in 25 kg (56 lb) blocks. Butter for immediate use should be stored refrigerated. For extended shelf life, butter should be stored frozen. To use butter as a concentrated source of milk fat, it requires melting and is considered a processing inconvenience.

In some parts of the world where refrigerated storage is at a premium, butteroil may be used by ice cream manufacturers as a concentrated source of milk fat. Butteroil is manufactured by removing moisture and residual serum solids from unsalted butter. Butteroil is sold in 55 gallon drums and is stored under ambient conditions. During storage, however, milk fat crystallizes and this may necessitate the warming of the oil prior to use in mix making. Butteroil is packed under nitrogen to delay the onset of rancidity.

All other things being equal, the preferred sources of concentrated milk fat (from most to least) are cream, unsalted butter, and butteroil. Choice depends upon availability, economics, local preferences, regulatory factors, and quality of the ingredients.

74.2.2 Concentrated Sources of Serum Solids

Milk solids-non-fat (MSNF) is skim milk solids and these are made up of lactose, protein, and milk salts. Proteins play an important role in emulsification of the fat. Milk proteins also help in developing overrun (aeration). The combination of emulsification and foaming create desirable texture. Proteins also contribute to the viscosity of the mix. Proteins are surface active agents (surfactants). Surfactancy of proteins results in desirable interfacial behavior.

74.2.2.1 Fluid Whole and Skim Milk. Both are excellent sources and should be used in the mix. However, because of their low serum solids content in contrast to the serum solids desired in ice cream mix, their use is limited. Skim milk should be purchased on the basis of a definite MSNF content in order to guard against dilution with water.

74.2.2.2 Plain Condensed Skim Milk. Fresh condensed skim milk is easy and convenient to use, has an excellent flavor. The concentrate may be paid for on the basis of the solids content, which runs around 25–30%. The heat treatment given fluid skim milk is usually the same as the regular pasteurizing range. The keeping quality of condensed skim milk is better than that of cream. It should be stored at 0–1°C and used while fresh and sweet (usually for 7–10 days).

74.2.2.3 Plain Condensed Whole Milk. This is concentrated about two and a half times and contains 8% fat and 20% serum solids.

74.2.2.4 Superheated Condensed Skim or Whole Milk. The use of superheated condensed milk may substitute the use of heat concentrated milk. The already-condensed product is slowly heated to a high temperature, usually in the range of 82.2°C. When properly done, a concentrate of much greater viscosity is obtained, which improves the whipping ability of the ice cream mix and contributes a smooth texture, which then binds more free water. Accordingly, less water is available to form ice crystals during freezing and shelf-life, and the smooth texture of the ice cream is maintained throughout its shelf-life. Superheating, therefore, functions like a stabilizer.

74.2.2.5 Sweetened Condensed Whole or Skim Milk. A sweetened condensed product may sometimes be used as a source of MSNF. This ingredient provides 8.5% fat and 28% total milk solids. The added sugar (40–44%) improves the keeping quality over that of plain condensed milk. With this concentration of sugar, the osmotic pressure of the solution is high enough to suppress the growth of practically all microorganisms. The product will keep at room temperature.

The titratable acidity test should be applied to all condensed milk products. When diluted so as to contain the same MSNF concentration as skim milk, the acidity should be approximately that of fresh skim milk (0.18%).

74.2.3 Balancing Ingredients

In order to balance a formula and make a mix, ingredients such as milk, skim milk, or water may be necessary. This is because a concentrated source of milk fat such as cream will contribute serum solids along with the fat. Similarly concentrated sources of MSNF may also contribute fat to the mix, for example, condensed whole milk. In instances where liquid sugar is used, water in the ingredient may dilute the solids. Therefore a balancing ingredient is necessary.

74.2.4 Sweeteners

Ice cream is a sweet frozen dessert. Its sweetness is due to the presence of sugars and other sweeteners. Sweeteners are classified either as nutritive or nonnutritive.

74.2.4.1 Nutritive Sweeteners. They provide four calories per gram of the sweetener and include sugar (sucrose, saccharose), lactose (milk sugar), dextrose (glucose), fructose (fruit sugar, levulose), corn syrup solids (glucose syrup solids), and high fructose corn syrups, sugar alcohols (xylitol, maltitol, sorbitol, and glycerol). A comparison of the properties of nutritive sweeteners is given in Table 74.5.

Sugar (sucrose). It provides sweetness, depresses freezing point, affects freezing performance, affects body and texture, enhances flavor, and contributes bulk or total solids and impacts on economics. Generally the equivalent of 15% sucrose is considered optimal sweetness in ice cream. In making no-sugar added ice cream and frozen desserts, the bulk contributed by sugar is absent and therefore bulking agents, such as polydextrose, are used. Sugars depress the freezing point of the ice cream mix.

TABLE 74.5 Comparison of Properties of Nutritive Sweeteners.

Sweetener	Relative Sweetness	Solubility (g/100 g) at 25°C	Chemical Type
Sucrose	1.0	67	Disaccharide
Glucose	0.6	51	Monosaccharide
Fructose	1.2–1.8	81	Monosaccharide
Invert sugar	1.0	–	Glucose and fructose
Lactose	0.3	16	Disaccharide
Sorbitol	0.6	72	Sugar alcohol
Mannitol	0.7	18	Sugar alcohol
Xylitol	1.0	64	Sugar alcohol
Corn syrup solids (36 DE)	0.45	70	Mixtures
Corn syrup solids (42 DE)	0.45	70	Mixtures
High fructose corn syrup	1.2	67	Mixture

Liquid Sugar. Liquid sugar is sugar syrup containing 67% sugar and 33% water. It is used by high volume ice cream manufacturers and is sold in rail tank cars or truck load quantities. It can be easily pumped and metered into ice cream mix making operations.

Monosaccharides. Glucose and fructose are simple sugars called monosaccharides. They depress the freezing point of water to a greater extent than disaccharides (sucrose, lactose). They are added as a part of the high fructose corn syrup mixture. High fructose corn syrup contains 45% fructose and 55% glucose and has the same sweetness as sugar. By further refining the proportion of fructose can be increased to 55% fructose, 45% glucose. The resulting product called high fructose corn syrup 55 is slightly sweeter than sugar. An additional refining step can increase the fructose content to 90%. This product is called high fructose corn syrup 90 and is approximately 1.8 times sweeter than sugar.

Sugar Alcohols. Sorbitol and xylitol are examples of sugar alcohols. They depress freezing point to an extent greater than disaccharides and similar to monosaccharides. Glycerol depresses the freezing point to a greater extent than sugar alcohols and ethanol (alcohol) depresses the freezing point to an extent greater than glycerol. These are illustrated in Table 74.6.

Corn Sweeteners. Often corn syrup solids or maltodextrins are added to ice cream mix formulations. Corn sweeteners are derived from the modification of corn starch. Low

TABLE 74.6 Effect of Nutritive Sweeteners on Freezing Point Depression.

Sweetener	Relative Effect
Sucrose	1.0
Lactose	1.0
Dextrose	1.82
Fructose	1.82
55% High fructose corn syrup	1.85
Sorbitol	1.90
Glycerol	3.70
Alcohol	7.40

conversion corn syrups are 23–38 dextrose equivalents (DE). DE is a measure of the extent of hydrolysis or modification of starch. Regular conversion syrups are 38–38 DE, intermediate conversion syrups are 48–58 DE, and high conversions are 58–68 DE. These products are not as sweet as sugar but they contribute total solids to the mix. By increasing total solids to the mix heat shock protection is provided.

Other Nutritive Sweeteners. Honey is used as a sweetener. Honey is like invert sugars. It is made up of glucose and fructose, both monosaccharides, therefore it depresses freezing point to a greater extent than sugar at equivalent concentrations.

Maltodextrins. In certain low fat and no fat mixes as well as no sugar added mixes, maltodextrins of 5 or 10 DE are used to provide solids in the mix without adversely affecting the freezing point of the mix. These products are also derived from corn starch and the modification of the starch is to a much lesser extent than with maltodextrins. Typically the DE ranges from 5 to 15 for maltodextrins.

74.2.4.2 Nonnutritive Sweeteners. No sugar added products are ice cream and frozen desserts in which no sugars are added to achieve sweetness. Such formulations rely on the addition of intense nonnutritive sweeteners. The intense sweeteners that do not provide any significant calories at use levels. They include sucralose, aspartame, saccharin, cyclamates, Acesulfame-K, and many others. As little as 0.07% aspartame can provide the sweetness equivalent to 15% sugar. By reducing the mass of the formulation by 14.93% we have more water in the formulation to control. In order to make up this difference bulking agents are used. One common bulking agent is polydextrose. Additionally, removal of sugar increases the freezing point of the mix. Therefore, to lower the freezing point sugar alcohols are used. A third adjustment necessary for no sugar added formulations is the increased levels of stabilizers in the formulation. A comparison of the non-nutritive sweeteners is provided in Table 74.7.

A typical formula for a no sugar added low fat ice cream would contain 3% fat, 12% MSNF, 8.0% polydextrose, 5% 10 DE maltodextrin, 1.2% microcrystalline cellulose, 0.35% stabilizer and emulsifier, 0.07% aspartame, and 2.0% sorbitol. The total solids would be 36.62%. This mix would freeze at 27°F (2.7°C).

74.2.5 Stabilizers and Emulsifiers

Apart from sweeteners, stabilizers, and emulsifiers are also important nondairy ingredients. The term stabilizer is used for a group of substances that help stabilize the structure

TABLE 74.7 Comparisons Among Nonnutritive Sweeteners.

Sweetener	Relative Sweetness	Solubility (g/100g) at 25°C
Saccharin	250–550	125
Cyclamate	30–50	Not known
Aspartame	120–200	1
Acesulfame-K	100–130	27
Alitame	2000	17
L-sugars	1.0	67
Sucralose	500–700	30

of ice cream. Other names include colloids, hydrocolloids, and gums, which indicate that these substances are large molecules (macromolecules) that are capable of interacting with water. By interacting with water also lets some of these compounds interact with proteins and lipids in the mix. A variety of materials are used as stabilizers. These include gelatin, guar gum, sodium carboxymethylcellulose, microcrystalline cellulose, locust bean gum (carob), and carrageenan. During mix processing, presence of gums affects mix viscosity and homogeneity, during freezing gums exert secondary effects in dryness and stiffness of ice cream and in the finished frozen desserts, it controls the properties of the water that is unfrozen. This last point means that ice cream is smoother and ice crystals take longer to grow in the presence of stabilizers especially during storage and distribution of these products. Usually, stabilizers are used at 0.1–0.5% levels in the mix but the actual amount depends on the type of stabilizer, strength of the stabilizer, total solids and fat level of the mix, duration and temperature of storage of ice cream and the method of pasteurization. High fat and high total solids mixes require lesser level of stabilizers. More stabilizer is needed for ice cream that is stored for a long period of time or if the temperature fluctuation during storage is frequent. If the mix is pasteurized by the high temperature short time method, more stabilizer may be needed than if the same mix were pasteurized by the batch method or by ultra high temperature method.

74.2.5.1 Stabilizers. A good stabilizer should be non toxic, readily disperse in the mix, not cause excessive viscosity, separation or foam in the mix, not clog strainers and filters, provide ice cream with good meltdown, be economical, and not impart off-flavor to the mix. Some of the common stabilizers and their characteristics are listed below.

Gelatin. It is an animal protein derivative and is effective at high concentrations of 0.3–0.5% and is expensive and therefore rarely used in the United States. It may not prevent the effects of heat shock. It is also not acceptable to certain religious and vegetarian segments of the population. If gelatin is used as a stabilizer, a long aging period for the mix is necessary. Gelatin disperses easily and does not cause wheying off or foaming.

Guar Gum. This stabilizer is derived from the seeds of a tropical legume called guar. It is the least expensive of the stabilizers and effectively mitigates the undesirable changes in ice cream due to heat shock. It readily disperses in the mix and does not cause excessive viscosity in the mix. Typically 0.1–0.2% is required in a mix and therefore this substance is considered to be a strong stabilizer.

Sodium Carboxymethyl Cellulose (CMC). This is a chemical derivative of cellulose. Cellulose is the most abundant carbohydrate in nature. CMC causes mix separation and therefore it is often blended with carrageenan to prevent wheying off. It is a strong stabilizer. Only 0.1–0.2% is need in a mix. It imparts body and chewiness to ice cream.

Locust Bean Gum. This is also derived from a plant seed and is also known as carob seed gum. It is a strong stabilizer and is used at 0.1–0.2% levels. Mix containing locust bean gum separates or wheys off during storage. It also does not fully hydrate in high temperature short time pasteurized mixes.

Carrageenan. This stabilizer is derived from a sea weed *Chondritis crispus*. It is used in many stabilizer blends at levels of 0.01–0.02%. This stabilizer reacts with milk proteins and thereby prevents wheying off in mixes.

74.2.5.2 Emulsifiers. As opposed to stabilizers, emulsifiers exert their action on the fat phase of ice cream. Emulsifiers are surface active agents (surfactant). Fat and water do not mix. Emulsifiers facilitate the mixing of fat and water because these molecules have two domains, one that likes water (hydrophilic), and another that likes fat (hydrophobic). When the hydrophobic part of a surfactant interacts with the fat, the water-loving part of the molecule can interact with water, thus facilitating the suspension of fat in water. Generally mono- and diglycerides and ethoxylated esters of sorbitol (polysorbates) are the commonly used emulsifiers. Mono - and diglycerides are derived from fatty acids and glycerol. Therefore, emulsifiers are fatty substances. They also show fat like properties of melting point, crystallinity, and they can be composed of saturated or unsaturated fatty acids. Presence of emulsifiers in ice cream leads to smoother texture and better shape retention, while improving the ability of the mix to incorporate air.

Mono- and Diglyceride Mixtures. These compounds are obtained by the chemical treatment of fats such as lard, palm kernel or soybean oil. Most of the mono- and diglycerides are solid at room temperature and are added to the mix prior to pasteurization at a level of 0.1–0.2%. Emulsifiers with high monoglyceride content are also effective drying agents.

Polysorbates. Polysorbates are polyoxyethylene compounds. These synthetic chemicals are the most effective drying agents. Polysorbate 80 is an oleic acid derivative. It is very powerful drying agent and is used at 0.04–0.07% levels. Polysorbate 65 is helpful as a whipping agent, that is, it helps in air incorporation. To obtain comparable stiffness, more polysorbate 65 has to be used than polysorbate 80. At high levels, polysorbate 80 imparts off-flavors whereas polysorbate 65 does not. Polysorbates are generally liquids and cause churning of the mix.

Egg Products. Dried or frozen egg yolks are used to produce dry, stiff ice cream. Dried egg yolks are harder to incorporate into a mix than frozen and sugared egg yolks. The general use level of egg yolks is 0.3–0.5%. If a French-style or custard is required, a minimum of 1.4% egg yolk solids are necessary. Lecithin, a phospholipid present in egg yolks is thought to act as the emulsifier. Lecithin can also be derived from soybean oil.

Buttermilk Powder. Buttermilk powder provides phospholipids which can act as emulsifiers.

74.3 MIX CALCULATIONS

Once a formula has been finalized, a recipe has to be created. Formulas specify composition of the desired mix in terms of percentages of fat, MSNF, sweeteners, stabilizers, and emulsifiers. A recipe calculates the weight and/or volumes of ingredients needed to meet the formula requirements. These calculations are called mix calculations.

Mix calculations are essential for manufacturing consistent quality finished products. When composition of raw materials varies or the economics of ingredients changes, the recipe for making an ice cream mix has to change. Further this change has to occur in a manner that the finished product composition is not altered. In some instances changing regulatory definitions and health claims may necessitate manufacture of products to carefully defined specifications. Mix calculations are important in standardizing mixes prior to

freezing. Ice cream plants now use computer software programs to calculate the amounts of various ingredients to conform to required specifications of composition of ice cream mix. However, for basic understanding, we will discuss below the fundamentals of the mix calculations.

Mix calculations can be performed by Pearson's Square, algebraic methods, and arithmetic methods. Pearson's Square is of limited utility, algebraic methods are complicated and involve the use of simultaneous equations and matrices. Arithmetic calculations are simpler and require fewer computations than algebraic method. In this section all three methods will be discussed. Prior to calculating a mix, certain preliminary steps have to be completed.

Several factors are important in mix calculations.

1. Composition of the mix has to be specified. Mix composition clearly states the percentage of fat, MSNF, sugar, other sweeteners, stabilizers and emulsifiers, and alternate ingredients. For example a typical mix composition would be milk fat 10.1%, MSNF 10%, sugar 8.0%, high fructose corn syrup 4%, corn syrup solids (36-DE) 5.0%, stabilizer, 0.3%, emulsifier 0.2%. The total solids of this mix are 37.6%. Although not specified explicitly, the water content of this mix is $100 - 37.6 = 62.4\%$.
2. The source of the ingredients should also be specified. For example milk fat will be derived from cream, MSNF from condensed skim milk, and so on.
3. The amount of mix to be made must also be determined. Generally if volumetric amounts are known (e.g., 100 L or 100 gallons), these quantities have to be converted to weight equivalents of kilograms or pounds. Specific gravity or density of the mix would have to be known.
4. In order to make an ice cream mix, three categories of ingredients are essential. These are (a) a concentrated source of milk fat, (b) a concentrated source of MSNF, and (c) a balancing ingredient.
5. In order to perform mix calculations, determine the composition of the mix to be made, list the available ingredients and their compositions, and list the amount of mix to be made in weight equivalents. Then perform calculations.
6. After performing calculations verify or validate the calculations by constructing a proof sheet.
7. Make the mix according to calculated amounts of the ingredients and test to make sure that the desired composition has been attained. If required, standardize the mix to obtain the desired composition.

74.3.1 Terms Used in Mix Calculations

Serum in dairy ingredients (milk, skim milk, cream, condensed skim milk, condensed whole, etc.) is the fat-free portion of that ingredient.

$\%$ Serum $= 100 - \%$ milk fat
$\%$ Serum $= \%$ MSNF $+ \%$ moisture
If fat is considered to be 0 in skim milk, serum in skim milk is $100 - 0 = 100$
Serum in 3.5% fat milk is $100 - 3.5 = 96.5$
Serum in nonfat dry milk (skim milk powder, assuming 0% fat) is $100 - 0 = 100$
Serum in sweetened condensed skim milk with 0% fat, 25% sugar is $100 - 25) = 75$

Serum in sweetened condensed whole milk with 8% fat and 40% sugar is $100 - (8 + 40) = 52$

Serum in ice cream mix is calculated slightly differently than serum in milk ingredients.

Percent serum in ice cream mix = 100 − (% milkfat + % sweeteners + % stabilizers/emulsifiers + other nondairy solids).

Serum in an ice cream mix with 10.1% fat, 10% MSNF, 8% sugar, 4% high fructose corn syrup, 5% corn syrup solids, and 0.3% stabilizer, and 0.2% emulsifier is: $100 - (10.1 + 8 + 4 + 5 + 0.3 + 0.2) = 100 - 27.6 = 72.4$.

Weight of product multi % of constituent as a decimal = weight of a constituent.

Example 1. Make 100 lb mix to contain 15% sugar, 0.35% stabilizer, 0.5% egg yolk solids, 12.5% fat, and 11% serum solids. Available ingredients are 40% fat cream, 3.5% fat milk, 8% fat, and 20% serum solids condensed milk.

Solution 2: For 100 lb mix, 15 lb sugar, 0.35 lb stabilizer, and 0.5 lb egg yolk solids are needed. Therefore $100 - (15 + 0.35 + 0.5) = 84.15$ lb has to be derived from cream and condensed milk.

Let $x = 40\%$ cream, $y = 3.5\%$ milk, and z = condensed milk. It follows that

$$x + y + z = 84.15 \tag{1}$$

We know that the total fat content must be 12.5 lb Therefore,

$$0.4\,x + 0.035\,y + 0.08\,z = 12.5 \tag{2}$$

Also we know that serum solids needed is 11% so

$$0.053\,x + 0.085\,y + 0.20\,z = 11 \tag{3}$$

Thus, we have three simultaneous equations for three unknowns. Eliminate an unknown by expressing in terms of other unknowns.

$$x + y + z = 84.15 \tag{1}$$

$$x = 84.15 - y - z \tag{1a}$$

Substituting this value for x in Eq. 2 we get.

$$0.40(84.15 - y - z) + 0.035\,y + 0.08\,z = 12.5 \tag{2a}$$

Simplifying Eq. 2a, it converts to

$$36.5\,y + 32\,z = 2116 \tag{2b}$$

Similarly Eq. 3 becomes

$$0.053(84.15 - y - z) + 0.085\,y + 0.20\,z = 11 \tag{3a}$$

Conversion of Eq. 3a results in

$$3.2\,y + 14.7\,z = 654.005 \tag{3b}$$

If we wish to eliminate one of the equations in (2b) and (3b) it is necessary that the multiples of unknowns which we wish to eliminate be the same in both equations. If we wish to eliminate y, then the multiples of y must be the same in both equations. This condition is obtained as follows:

Multiply Eq. 2b by 3.2 to obtain Eq. 2c and multiply Eq. 3b by 36.5 to obtain Eq. 3c.

$$116.8\,y + 102.4\,z = 6771.2 \tag{2c}$$

$$116.8\,y + 536.55\,z = 23871.18 \tag{3c}$$

Subtract Eq. 3c from Eq. 2c and the result is:

$$434.15\,z = 17099.98 \tag{4}$$

$z = 17099.98$, $434.15 = 39.39$ lb of condensed milk.

Solve for y by substituting this value for z in either Eq. 2b or Eq. 3b. Substituting in Eq. 2b we get:

$$36.5\,y + (32 \times 39.39) = 2116$$

$$36.5\,y = 855.52$$

$y = 855.52$ 36.5 = 23.44 lb 3.5% fat milk.

By substituting found values of y and z in Eq. 1 we get:

$$x + 23.44 + 39.39 = 84.15$$

$x + 84.15 - (23.44 + 39.39) = 84.15 - 62.83 = 21.32$ lb cream.

To validate the calculation let us construct a proof table.

Weight	Ingredient	Fat	Serum Solids
15.0	Sugar	0.00	0.00
0.035	Stabilizer	0.00	0.00
0.50	Egg yolk solids	0.00	0.00
21.32	Cream	8.5280	1.1299
23.44	Milk	0.8204	1.9924
39.39	Condensed milk	3.1512	7.8780
100.00	Total calculated	12.4996	11.0003
100.00	Total desired	12.5000	11.0000

74.3.2 Arithmetic Method

This method relies on two main types of equations. One is called the serum point formula and is useful when fat is derived from only one ingredient. The other is called milk and cream formula and is used when multiple sources of fat are involved.

74.3.2.1 Serum Point Formula.

This equation is used to calculate the amount of concentrated source of serum solids need for a mix recipe.

$$\frac{\text{lb serum solids needed} - (\text{lb serum of mix}) \times 0.09}{\text{lb serum solids of 1 lb condensed} - (\text{lb serum of 1 lb condened}) \times 0.09}$$

The terms serum solids needed are given in mix composition, the word condensed simply means concentrated source of MSNF. This equation contains both terms *serum* and *serum solids*.

74.3.2.2 Milk and Cream Formula

$$\frac{\text{lb fat needed} - (\text{lb milk} + \text{cream needed}) \times (\text{lb fat of 1 lb milk})}{\text{lb fat of 1 lb cream} - \text{lb fat of 1 lb milk}}$$

The milk and cream formula is used in instances where fat is supplied by more than one source in the same recipe.

1. A 1000 lb ice cream mix is composed of the following ingredients:

Ingredient	*Lb*
40% fat cream	237.3
4% fat milk	376.9
29% SS/MSNF condensed skim milk	222.8
Sugar	160.0
Stabilizer/emulsifier	3.0

   (a) Enter the pounds of each ingredient into a proof table.
   (b) Calculate the pounds of each constituent contributed by each ingredient. Constituents are fat, MSNF, sugar, stabilizer/emulsifier.
   (c) Total the pounds in each of the constituent columns of the proof sheet and calculate the percentage composition to the nearest hundredth percent of each of the mix constituents.

Answers:

a. Pounds of milk fat from cream = 237.3 × 0.40 = 94.92 lb
b. % Serum solids in cream = 100 − 40 = 60 × 0.09 = 5.4%
c. Pounds of serum solids in cream = 237.3 × 0.054 = 12.81 lb
d. Pounds of total solids in cream = 94.92 + 12.81 = 107.73 lb
e. Pounds of fat from milk = 376.9 × 0.04 = 15.08 lb
f. % Serum solids in milk = 100 − 4 = 96 × 0.09 = 8.64%
g. Pounds of serum solids in milk = 376.9 × 0.0864 = 32.56 lb
h. Pounds of total solids in milk = 15.08 + 32.56 = 47.64 lb
i. Pounds of milk fat in condensed skim milk = 0.0
j. Pounds of serum solids in condensed skim milk = 222.8 × 0.29 = 64.61 lb
k. Pounds of total solids in condensed skim milk = 64.61 lb
l. Pounds of sugar = 160 lb

m. Pounds total solids of sugar = 160 lb
n. Pounds of stabilizer/emulsifier = 3 lb
o. Pounds of total solids of stabilizer/Emulsifier = 3 lb
p. Total pounds of mix = 237.3 + 376.9 + 222.8 + 160 + 3.0 = 1000
q. Total pounds of milk fat = 94.92 + 15.08 = 110.0
r. % Milk fat = 110/1000(100) = 11.0%
s. Total pounds of serum solids = 12.81 + 32.56 + 64.61 = 109.98
t. % Serum solids = 109.98/1000(100) = 10.998 or 11.0%
u. Total pounds of sugar = 160
v. % Sugar = 160/1000(100) = 16%
w. Total pounds of stabilizer/emulsifier = 3
x. % Stabilizer/emulsifier = 3/1000(100) = 0.3%
y. Total pounds of solids in mix = 382.98
z. % Total solids of mix = 382.98/1000(100) = 38.298 or 38.3%

74.3.3 Mix Proof Sheet

Desired Formula		Available Ingredients
___% Milk fat	237.3 lb	40% fat cream
___% Milk solids not fat	376.9 lb	4% fat milk
___% Sucrose	222.8 lb	Condensed milk 29% MSNF (SS)
___% Corn syrup solids	160.0 lb	Sugar
___% Stabilizer	3.0 lb	
Stabilizer/emulsifier		
___% Emulsifier		
___% Total solids		
___Lb of mix		

Ingredient	Quantity (lb)	Milk Fat (lb)	Serum Solids (lb)	Sucrose (lb)	Corn Syrup Solids (lb)	Stabilizer (lb)	Emulsifier (lb)	Total Solids (lb)
40% fat cream	237.3	94.92	12.81					107.73
4% fat milk	376.9	15.08	32.56					47.64
29% MSNF condensed milk	222.8	0.0	64.61					64.61
Sugar	160.0			160.0				160.0
Stabilizer/ emulsifier	3.0					3.0		3.0
Total	1000.0	110.0	109.98	160.0		3.0		382.98
Percent	100	11.0	11.0	16.0		0.3		38.3

Example 2. Calculate the amount of ingredients needed to make 100 lb of an ice cream mix:

Desired Composition	Available Ingredients
10.00% Milk fat	30% fat cream
12.00% MSNF	Skim milk
15.00% Sugar	Condensed skim milk (27%MSNF)
0.30% Stabilizer/emulsifier	Sugar
37.3% Total solids	Stabilizer/emulsifier

Fill in the attached proof sheet and provide all calculations supporting your solution.

a. Pounds of serum solids needed $= 100 \times 0.12 = 12.0$
b. Pounds of serum of mix $= 100 - (10 + 15 + 0.3) = 100 - 25.3 = 74.7$
c. Pounds of serum solids of 1 lb condensed $= 27/100 = 0.27$

$$\frac{\text{lb serum solids needed} - (\text{lb serum of mix}) \times 0.09}{\text{lb serum solids of 1 lb condensed} - (\text{lb serum of 1 lb condened}) \times 0.09}$$

d. Pounds of condensed skim needed $= \{12 - (74.97 \times 0.09)\}/\{0.27 - (1.0 \times 0.09)\} = (12 - 6.723)/(0.27 - 0.09) = 29.32$
e. Pounds of cream needed $= 10/0.3 = 33.33$
f. % serum in 30% cream $= 100 - 30 = 70$
g. % Serum solids in cream $= 70 \times 0.09 = 6.3$
h. Pounds serum solids in cream $= 33.33 \times 0.063 = 2.09979$ or 2.10
i. Pounds of skim milk required (by difference) $= 100 - (33.33 + 29.32 + 15.0 + 0.3) = 22.05$
j. Pounds serum solids in skim milk $= 22.05 \times 0.09 = 1.9845$ or 1.98
k. Pounds of sugar needed $= 100 \times 0.15 = 15$
l. Pounds of stabilizers/emulsifiers needed $= 100 \times 0.003 = 0.3$

Desired Formula	Available Ingredients
10.0% Milk fat	30% fat cream
12.0% Milk solids not fat	Skim milk
15.0% Sucrose	27% MSNF condensed skim
0.0% Corn syrup solids	Sugar
0.3% Stabilizer/% emulsifier	Stabilizer/emulsifier
37.3% Total solids	
100 Lb of mix	

Ingredient	Quantity (lb)	Milk Fat (lb)	Serum Solids (lb)	Sucrose (lb)	Corn Syrup Solids (lb)	Stabilizer (lb)	Emulsifier (lb)	Total Solids (lb)
30% fat cream	33.33	10.0	2.10					12.10
Skim milk	22.05		1.98					1.98
27% MSNF condensed skim	29.32		7.92					7.92
Sugar	15.0			15.0				15.00
Stabilizer/ emulsifier	0.3					0.3		0.30
Total	100.0	10.00	12.00	15.00		0.30		37.30
Percent	100	10	12	15		0.3		37.30

74.4 PROCESSING

Figure 74.1 shows various steps involved in the manufacture of ice cream. Knowing a mix specification, mix calculations are performed to determine the amounts of desired ingredients needed to formulate the mix. Mix processing begins with the assembly of the necessary ingredients in the desired amounts. Generally this assembly requires weighing the ingredients or if liquid ingredients are used they are metered. Meters rely on knowing the density or the specific gravity of the ingredient and these values are highly temperature dependent. In most small scale operations weighing is the method of choice.

74.4.1 Blending

In the next step the ingredients are blended together. Mix blending can be performed at refrigeration temperatures 40°F (4°C) or at warmer temperatures 113°F (45°C). Cold batching is preferred method when cream, liquid milk, condensed skim are the ingredients. Warmer temperatures are generally used when the ingredients include butter, butteroil/ anhydrous milkfat in combination with nonfat dry milk. Batching begins by placing a liquid ingredient in a vat. Generally this ingredient is skim milk (balancing ingredient), or water. The dry ingredients such as corn syrup solids, maltodextrins, sugar, stabilizers, and emulsifiers are incorporated into this liquid and finally cream is added. When cream is subjected to excessive shear it can be churned to butter. This should be avoided. Incorporation of the dry ingredients is aided by one of two types of devices, namely, (a) powder horn, and (b) high shear mixers. The powder horn is the easiest device and consists of a funnel with a valve placed in line with a pump and capable of recirculating the liquid in the vat (Fig. 74.2).

With the valve closed, the dry ingredient is placed in the funnel, the pump is started and once the fluid is recirculation, the valve under the funnel is opened. As the liquid flows past the funnel it inspires (sucks in) the powder. The mixture of the powder and the liquid hits the impeller of the centrifugal pump which facilitates the dispersion of the powders. The dispersed powder enters the vat and is recirculated. This process continues until such time

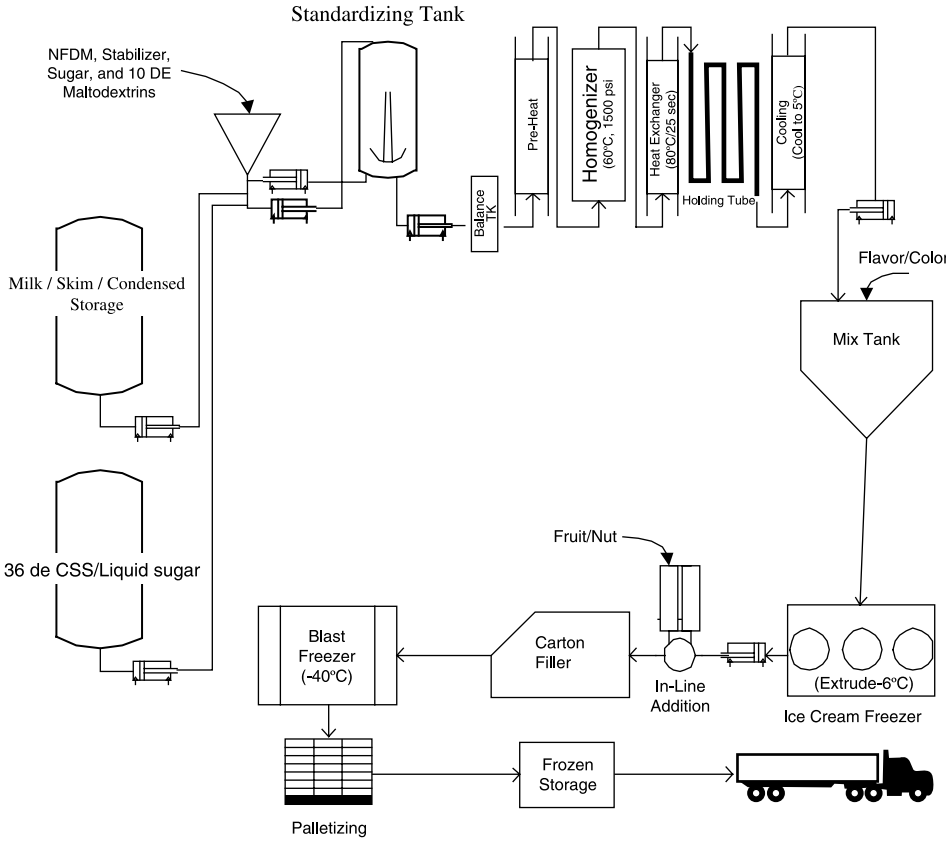

Figure 74.1 *Flow sheet diagram for the manufacture of ice cream.*

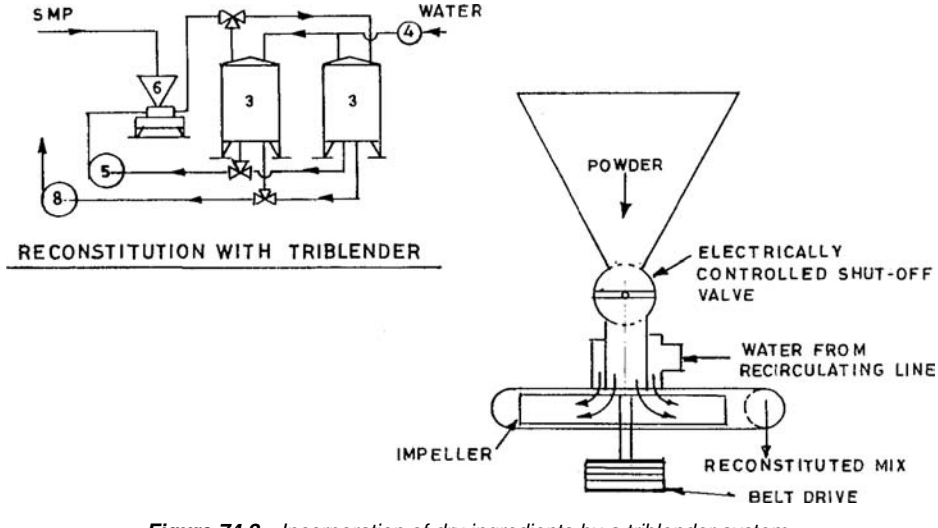

Figure 74.2 *Incorporation of dry ingredients by a triblender system.*

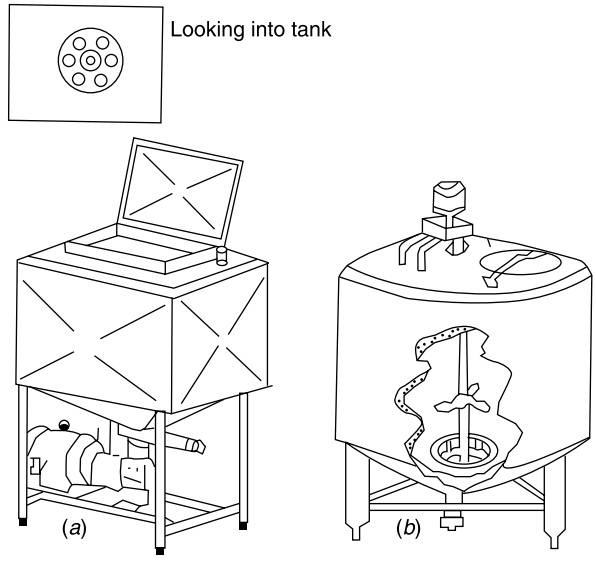

Figure 74.3 *High shear mixing devices (a) Likwifier and (b) turbine blender.*

that all of the powder has been incorporated into the process fluid and then the valve beneath the funnel is closed. If this valve is not closed a large amount of air can be inspired into the mix creating foam which is undesirable.

In the second process (Fig. 74.3) a high shear mixer that functions like a giant Waring blender is used.

Here the process fluid is filled to three-quarters of the volume of the blender. The motor is turned on and under vigorous agitation the dry ingredients are incorporated into the mix. Once all the dry ingredients are incorporated, the mixture is discharged into a vat. The hardest ingredients to incorporate are the stabilizers and emulsifiers. If they are not properly handled they form lumps and are not uniformly dispersed in the mix. Excess agitation is undesirable in suspending these ingredients. Generally, mixing stabilizers with dry sugar and corn syrup solids aids in a uniform hydration and suspension of these ingredients.

74.4.2 Pasteurization

The hydrated ingredients are pasteurized and homogenized. Pasteurization is a heat treatment given to food products to destroy pathogenic (disease causing) microorganisms. According to the U.S. Public Health Service and its Pasteurized Milk Ordinance, pasteurization of an ice cream mix requires that every drop of mix be heated to 155°F (68.3°C) and held at that temperature for 30 min. Alternately, every drop of mix should be heated to 175°F (79.4°C) and held at that temperature for 25 s. Pasteurization can be performed either as a batch operation or as a continuous operation.

The batch pasteurization is carried out in a specially designed and approved vat. A batch of mix is placed in the vat and pasteurized by heating the mix to a minimum of 68.3°C (155°F) and once that temperature is attained, it is held for 30 min prior to homogenization and cooling. This process is also known as the low-temperature-long-time (LTLT) method of pasteurization.

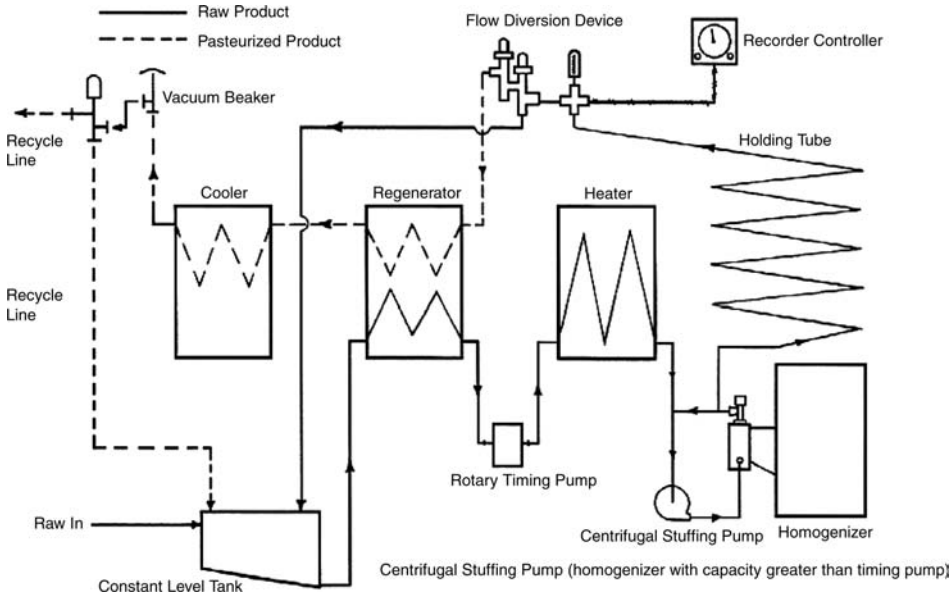

Figure 74.4 *Schematic of a high temperature short time pasteurizer with timing pump and homogenizer.*

In the continuous process, a plate heat exchanger is used. This heat exchanger has three sections called (a) regeneration, (b) heating, and (c) cooling sections. In this process the mix is heated to a minimum temperature of 79.9°C (175°F) and held for 25 s; raw cold mix enters the regeneration section where it is heated by the hot pasteurized mix. The warm raw mix is then homogenized and heated by hot water to 79.9°C (175°F). The heated mix flows through a tube called the holding tube for 25 s. Then it has to pass through two controls known as the flow diversion devices. If the mix is cooled to below 79.9°C (175°F) during the holding, these flow diversion devices sense this and send the mix to be repasteurized. Once the mix successfully flows past the flow diversion devices, it enters the regeneration section where it gives off some of its heat to the incoming raw mix. Thus the regeneration section is an energy saving device. The pasteurized side of the plate is maintained at a minimum pressure differential of 2 psi so that raw mix cannot contaminate the pasteurized mix. The partially cooled mix then goes to the cooling section where it is cooled to 4°C (40°F). One schematic of a continuous pasteurizer also called a high temperature short time (HTST) is shown in Figure 74.4.

74.4.3 Homogenization

The process of homogenization has been referred to during the pasteurization step. Homogenization of an ice cream mix results in a smoother eating ice cream. It is a process whereby fat droplets in the mix are reduced to a uniform size. In unhomogenized ice cream mix, the average fat droplet size would be around 2–4 μm. Homogenization breaks down the fat globules to an average size of 1 μm or less. One micrometer is 1/25,000th of an inch, a size not visible to the naked eye. In order to homogenize the ice cream mix, all the fat must be in the liquid state. Therefore homogenization is performed

on hot mix. In a LTLT (batch) pasteurization system, the homogenization is done after the heating and holding of the mix at a temperature of around 68.3°C (155°F). In the HTST system of pasteurization, homogenization can be performed either after the mix is warmed up in the regeneration section where mix temperature is around 62.7°C (145°F) or after the mix is pasteurized prior to entering the regeneration section when the temperature is 79.9°C (175°F). It is preferable to homogenize prior to pasteurization. The typical pressures for homogenization are 2000 psi first stage and 500 psi second stage. In high fat mixes, high acid mixes, chocolate mixes, and mixes with high amounts of egg yolk, homogenization pressures are reduced to 1200 psi first stage and 500 psi second stage.

74.4.4 Aging

The pasteurized, homogenized mix then is aged in a refrigerated vat. Aging is a process of quiescent storage of the mix with intermittent agitation for a period varying from 3 h to 16 h. During the aging process, the fat crystals that melted during pasteurization recrystallize, the gums or stabilizers also complete their hydration process and the proteins complete their adsorption at the fat/water interface. In the days when gelatin was used as the primary stabilizer in ice cream, aging times of 12–20 h were recommended. Modern day stabilizers do not use gelatin and require far less time to complete their hydration. A minimum aging time of 2–4 h is recommended.

74.4.5 Flavors

Flavor is the most important esthetic attribute of a food and ice cream is no different. Ice cream differs from other food products in that it has no preconditioning flavor like some other foods do. The flavor of ice cream will only become apparent when the product is in the mouth and undergoes melting. You can smell ice cream and not be able to discern the flavor of the frozen product. The term flavor is composed of two important attributes, namely, taste and aroma. In the tasting of ice cream, all five senses are used. The sense of sight is used to determine the color of the product, homogeneity of the product and sometimes one may observe ice crystals at the surface. The sense of touch is employed because when ice cream enters the mouth one can sense its temperature that it is cold and smooth. The sense of hearing may also be employed as the ice cream is moved around in the mouth and masticated sound travels along the jawbone to the ear canal and such things as crunchiness of ice crystals can actually be heard. The other two senses, smell and taste are the basis of flavor experience. The flavor of ice cream can be treated analogous to the ubiquitous computer. Sensory evaluation involves an input in the form of stimulation. The brain is the microprocessor that transforms these sensations and the out put is the reaction of like, dislike and so on. Since the subject has psychological and physiological variations, the response is not as reproducible as that of an instrument. Therefore, statistical techniques are employed to understand the consistency of reactions of the fickle instrument called the human brain. This chapter will not delve into the intricacies of sensory evaluation.

Flavor is an important attribute of a food. It is a sensory response that has three components, olfaction (odor/smell), gustation (taste), and tactual (mouthfeel). Ice cream is cold, creamy, refreshing, and sweet, and releases aroma upon melting in the mouth. When the word "flavor" is used in everyday parlance we imply taste and olfaction. Taste compounds are sweet, salty, bitter, and sour. Generally compounds imparting

these tastes can be detected at levels of 0.01–0.5. Olfactory compounds (smelly stuff) are volatile and have thresholds in the parts per million to parts per trillion range. Threshold is defined as the minimum concentration that at least 50% of the population can detect. Threshold of aroma compounds are 10 to 10 million times less than taste compounds.

Perception of aroma is affected by the composition, physical structure and temperature of the food. Undesirable flavors are called off-flavors. Off-flavors affect the overall flavor qualities of the food. Deteriorative reactions are time dependent and cumulative. Therefore, the length and conditions of storage has a profound influence on the perception of overall flavor. These deteriorative reactions occur in ingredients used in ice cream manufacture. Therefore, careful attention should be paid to the quality of ingredients used in ice cream manufacture.

In eating ice cream, whether you lick, bite, or chew, ice and fat melt. Melting of these two constituents leads to the collapse of the air cell. Upon collapse of the air cell, flavor volatiles are released. Flavor volatiles traverse the palette and enter the olfactory membrane. The brain then recognizes the signal and processes it. An ice cream mix is compounded and processed to obtain a neutral flavored base. This base has the ability to acquire any characterizing flavor added to it. This neutrality of flavor also means that if the mix is mishandled it can easily absorb off flavors. The ice cream mix contains fat, MSNF, sweeteners, stabilizers, and emulsifiers, and in some instances other additives. The preferred source of fat is milk fat and the flavor quality of cream and milk should be carefully monitored. If milk fat is improperly handled, off-flavors can easily result. When the source of fat is other than milk, other flavors may be present. Most flavor compounds are fat soluble. Milk solids-not-fat sources contribute a slightly salty note but can also contribute to stale, caramelized, old ingredient flavor notes. Off-flavors from whey solids and buttermilk solids should be avoided by using fresh supplies of these ingredients. The most common sweetener used is sugar. Sugar helps augment certain flavors. Corn syrup solids and high fructose corn syrup solids are also used as sweeteners. These could contribute a syrupy flavor and may mask the delicate flavor notes of some other ingredients. Mix processed in batch processors is particularly prone to this syrupiness. Stabilizers rarely pose flavor problems but by increasing the viscosity may slow the release of delicate flavors. Emulsifiers rarely pose flavor problems unless they are old. Rotating stocks and inventory control can avoid these problems.

Determining how much flavoring to add is always important. Know what your customers like. There are regional preferences for flavors and intensities of flavors. As a rule of thumb, higher the fat content of the mix, more the flavoring required. Also batch pasteurized mixes, especially if they contain egg yolk solids, require more flavoring HTST pasteurized mixes. Always test the effectiveness of the added flavor in the ice cream and not by judging the quality of flavored mix.

Flavors are added in at least three different ways, namely, (1) directly to the mix prior to freezing (e.g., vanilla, chocolate, mint), (2) immediately post freezing (fruit pieces, nuts, candy, and confectionery pieces); or (3) postfreezing prior to packaging (ripples and variegates). Modern flavoring systems are complicated and may use all three of these modes of flavoring in the same ice cream. The most popular flavors are vanilla, chocolate, fruits, nuts, bakery goods, confectionery items, and ripples or variegates. Nearly 30% of the ice cream manufactured is vanilla.

Vanilla is a standardized food. It is the only flavoring substance to be so classified. According to the Food and Drug Administration, single fold vanilla extract must contain 13.8 oz vanilla bean material in 70 proof alcohol. Ice cream made with pure

vanilla extract is labeled Category I as vanilla ice Cream. Ice cream flavored with a mixture of vanilla extract and vanillin (a mixture of natural and artificial with the natural predominating) is labeled as Category II ice cream or vanilla flavored ice cream. The third type is called Category III and consists of any vanilla that is not Categories I and II. Such a product is labeled as artificially flavored vanilla ice cream.

Vanilla is the bean of the only edible orchid *Vanilla plantifolia Andrews*. The bean only grows around the equatorial belt. Mexico, Madagascar, India, Indonesia, and The Comoros are all good growing areas for the vanilla orchid. The beans produced from the orchid are cured and shipped to countries where the flavor is extracted using alcohol and water.

Chocolate is another popular flavor. Chocolate is derived from the beans of a plant called *Theobroma cacao*. This plant also grows in the equatorial regions of the world and countries. Ghana, Ivory Coast, Brazil, Cameroon, Indonesia, Malaysia are all noted for the production of cocoa beans. Beans contain fat and other materials. Once fat is extracted cocoa powder is the residue. Cocoa powders can either contain 10/12% fat or 22/24% fat. Cocoa powders still contain fiber which can be removed by alkalizing the powder. This process is also known as "Dutch Cocoa." The most common flavoring material is 10/12 cocoa for light, low and no fat ice creams whereas 22/24% cocoa is used for regular ice cream.

Fruit flavors are popular and can be added as extracts, essences often with other natural flavors (WONF). Fresh fruit of good quality can also be added and slightly overripe fruits are preferred for this purpose. However fruits are seasonal horticultural products. The allure of fruit flavored ice cream is to eat it when the fruits are not in season. To accomplish this fruits are sliced and packed with sugar and frozen. Generally one part of sugar is added to three parts of fruit (three plus one pack) or one part of sugar per four parts of fruit (four plus one pack). Sugar is added to protect the fruit during the freezing process. The frozen fruits have to be thawed prior to adding to ice cream. Some fruits such as, strawberries, cherries, and pineapple can be heat treated and the flavor improved due to the heat treatment. Stabilized packs need not be refrigerated and some stabilized fruits may have a jam-like flavor rather than that of the fresh fruit.

Nuts like pecans, almonds, walnuts, cashews, hazelnuts, peanuts, macadamia, and pistachios are also used to flavor ice cream. Nutmeats must be free of shells, clean, fresh (free of rancidity) and should have low microbial counts. Nuts are generally roasted and salted to keep them fresh. Nuts contain a large amount of unsaturated fatty acids which are susceptible to rancidity. The best results are obtained by using fresh roasted and salted good quality nuts.

Ripples or variegates are a method of flavoring ice cream which incorporates unusual appearance and flavor into ice cream. A good ripple is soft flavorful and distinctive. Sugar present in the ripple may affect the freezing and storage characteristics of ice cream. Most ripples have stabilizers to impart viscosity. Ripples are introduced into the product by one of two methods: (a) freezer whipping or (b) pumps other than the freezer. Whipping in the freezer involves double duty for the equipment. The freezer first freezes the ice cream and is then slowed down to incorporate 10–12% of variegating sauce. Air actuated pumps are also used to pump the ripple sauce into the ice cream just prior to packaging. Variable speed controls on such pumps allow different amounts of ripple sauce to be deposited into the product. Ripples can have been of such varied flavors as chocolate, marshmallow, peanut butter, butterscotch, caramel, fudge, raspberry, blueberry, or other fruits.

Candy and confectionery pieces have become popular flavoring materials in ice cream. Toffees and hard candies are popular flavors. Hard candies have a moisture content of $<2\%$ and need to be stored properly in order for them to be added without difficulty into ice cream. In the ice cream these pieces should have a clean bite rather than a sticky, tacky one. The candy pieces must be sufficiently large to retain their piece identity in ice cream.

Baked pieces like cookies, cookie dough, cakes, pie crusts, and so on, are also used as flavorings. Over a period baked goods absorb moisture from the ice cream and become soggy and lose their freshness. Some baked items are fragile and end up as dust in the product. This is not desirable.

The final point about flavorings is that it is the first bite or lick that hooks the consumer if the flavor is good. Therefore flavor is one of the most important attributes of ice cream. When purchasing flavors, do not be influenced by the cost per unit of the additive but rather by the impact and quality of the impact that results from this ingredient. Calculate the cost of the flavoring on a use basis rather than the cost of the ingredient per se.

74.4.6 Freezing and Hardening

When freezing of ice cream is discussed, it is important to remember that it concerns the creation of ice from water in the mix. Therefore, the only constituent of the mix being frozen is water. During the freezing process the equilibrium between water and ice is altered. Freezing is facilitated by the removal of heat from a substance. In the old salt and ice machine, used prior to mechanical refrigeration, ice served as the refrigerant and addition of salt lowered the freezing point of water. The brine extracts heat from the mix. The mix temperature is lowered and the brine temperature increases. Brine is not a good refrigerant. With the advent of mechanical refrigeration, the use of ice and salt for freezing ice cream was relegated to a hobby status.

74.4.6.1 Refrigeration. Sensible heat is the heat which when added or removed causes a change in temperature of the product. Sensible heat can be measured by observing the temperature of the substance. Latent heat (hidden heat) is the heat required to bring about a change of state. For example to convert water to ice at $0^{\circ}C$ ($32^{\circ}F$) requires the removal of a large amount of heat and latent heat cannot be observed by temperature changes.

Mechanical refrigeration relies on fluids that have a low boiling point and a high latent heat of vaporization. In the vapor phase the refrigerant must be dense, nontoxic, low flammability, immiscible with oil, and low cost. Ammonia is the commonly used refrigerant in large installations.

Mechanical refrigeration units rely on four elements: (1) evaporator, (2) compressor, (3) condenser, and (4) expansion valve. The refrigerant circulates between these four elements and changes state from liquid to gas to liquid. In the evaporator the liquid refrigerant evaporates under reduced pressure and in doing so absorbs latent heat of vaporization and cools the medium being frozen. The other parts of the refrigeration recycle the refrigerant. Refrigerant vapor passes from the evaporator to the compressor where the pressure is increased. The high pressure vapor then passes to the condenser where it is condensed to a high pressure liquid. The liquid passes through an expansion valve where pressure is reduced and the cycle starts again.

74.4.6.2 Freezing Ice Cream. This refrigeration cycle is responsible for freezing water in an ice cream mix. Latent heat of crystallization for water is 1 kilocalorie per kilogram or 144 British Thermal Unit (BTU) per pound. The freezing point of a food is the temperature at which a minute crystal of ice exists with the surrounding water. However, before an ice crystal can form, a nucleus of water molecules must be present. Nucleation therefore precedes ice crystal formation. In the freezing of an ice cream mix the freezing point is determined by the amount and types of solutes present. The important solutes are sugars and milk salts and any other dissolved low molecular weight materials. In this regard, it must be borne in mind that monosaccharides depress freezing point to a greater extent than disaccharides and salts depress freezing point two to three times greater than an equal concentration of sugars.

As the refrigeration is turned on and the mix is agitated, it soon reaches its freezing point and nucleation takes places, followed by freezing of some water. Once some of the water is converted to ice, the concentration of the solutes increases and a new freezing point is established. The refrigerant removes some more heat and the new freezing point is reached, nucleation occurs and some more water is frozen. Once again, the concentrations of the solutes increase and yet another freezing point is established and the process is continued until the desired amount of water is frozen. Because this water is frozen rapidly and under agitation small ice crystal nuclei are formed. In the freezing of an ice cream mix, approximately 50% of the water in the mix should be frozen as quickly as possible (matter of minutes). The freezing of half of the water in the mix in a rapid manner results in a large number of small ice crystals. This is desirable in creating a smooth textured ice cream.

74.4.6.3 Overrun. In addition to the freezing process agitation helps in the incorporation of air into the ice cream. Incorporation of air leads to a volume expansion of ice cream. The term overrun is used to describe the increase in volume of the ice cream. Thus if 1 gallon (3.75 L) of mix is converted to 2 gallons of ice cream (7.5 L) we have effectively doubled the volume of the mix. This is termed 100% overrun. Overrun can be calculated on a volume basis or on a weight basis as follows:

On a volume basis

$$\text{Percent overrun} = \frac{\text{Volume of ice cream made} - \text{Volume of mix used}}{\text{Volume of mix used}} \times 100$$

On a weight basis

$$\text{Percent overrun} = \frac{\text{Weight of unit mix} - \text{Weight of equal volume of ice cream}}{\text{Weight of equal volume of ice cream}} \times 100$$

Example 1: 500 gallons of mix were used to produce 850 gallons of ice cream. The overrun would be calculated as follows: $\{(850 - 500)/500\} \times 100 = (350/500) \times 100 = 0.7 \times 100 = 70\%$.

Example 2: A mix weighs 8.9 lb to a gallon and the finished ice cream weighs 4.5 lb to a gallon. The overrun would be calculated as follows: $\{(8.9 - 4.5)/4.5\} \times 100 = (4.4/4.5) \times 100 = 0.978 \times 100 = 97.8\%$.

In practical ice cream operations, a target overrun is chosen for the product and then package weights are calculated. For example, it is desired to make 85% overrun ice cream and a gallon of mix weighs 9.1 lb the gallon of 85% ice cream is calculated by the formula:

$$85\%\ \text{overrun} = \frac{9.1 - x}{x} \times 100$$

Or

$$\frac{9.1}{1.85} = 4.92\,\text{lb}$$

from this calculation the weight of a half gallon of this ice cream can be set at 2.46 lb and a quart of this ice cream should weigh 1.23 lb and a pint of this ice cream should weigh 0.61 lb or 9.8 oz.

74.4.6.4 Types of Ice Cream Freezers

Batch Freezer. Generally ice cream can be frozen in a batch or a continuous mode. Batch freezers are commonly used by small ice cream shops that make ice cream on the premises. In batch freezers a predetermined amount of mix is charged into the freezing chamber, refrigeration is turned on as is the agitation. Generally the mix will occupy half of the barrel. The mix is agitated and whipped while being cooled. After some time the mix begins to freeze and when it achieves a certain consistency it begins to incorporate air. Incorporation of air in conjunction with the freezing stiffens the ice cream. At this point the refrigeration should be turned off and agitation continued for some additional period of time. When the desired overrun is achieved, the ice cream is discharged from the barrel with the agitator mechanism still on. Just prior to discharge of the ice cream, fruits and nuts can be added to the barrel but the preferred method of addition of particulate inclusions is to fold it in to the ice cream as it is being discharged from the barrel. Once this process is complete, the next batch of mix can be charged into the freezer barrel and the process repeated. The important variables are the composition of the mix, temperature of the mix, desired overrun, refrigerant temperature, the type and model of the freezer, and condition of the scrapper blades in the agitation mechanism (dasher). Under ideal conditions a batch of mix should be frozen in 8–10 min. There is some skill to operating such a freezer and batch to batch variations are routine in such products.

Continuous Freezer. Continuous freezers are commonly used in larger ice cream manufacturing plants where more than 500 gallons (1875 L) of ice cream per day may be manufactured. Continuous ice cream freezers have larger capacities, can be operated continuously, ingredients can be added in-line and packaging can be also automated. Also, continuous freezers make it possible to produce ice cream of different shapes through extrusion devices. Novelty extrusions such as sandwiches, prefilled cones and cups, cakes, and so on, are possible through the use of continuous freezers. The ice cream from a continuous freezer is smoother and creamier than a product from a batch freezer. This is because the ice crystals formed in a continuous freezer are smaller and the air cells may also be more uniform. The ice cream exiting a continuous freezer is also generally colder than that coming out of a batch freezer. There are a number of different types of continuous ice cream freezers, some are vertical freezers, especially for

smaller scale operations, others are horizontal ice cream freezers. Regardless of whether the freezing cylinder is horizontal or vertical all continuous freezers have a set of blades for scrapping the walls of the freezers. In a continuous freezer a mixture of air and mix is introduced at one end and is progressively frozen until ice cream is discharged at the other end. The conveyance of the mixture of air and mix and the discharge of the ice cream may be facilitated by coordinated pumps in some models. Also the newer models of freezers are equipped with microprocessor controls that monitor and control the discharge temperature of the ice cream, the viscosity of the ice cream and the overrun of the ice cream. Further these microprocessors can work in tandem with other downstream equipment such as ingredient feeders and packaging lines.

In continuous freezers the air for the overrun has very little effect in the freezing cylinder because it is compressed. In a freezer operating with 4 atm cylinder pressure, the air required to give 100% overrun occupies only 15% of the volume of the total mix. The density of the mixture in the freezer is not altered enough by the air to affect the rapid internal heat flow to the cylinder walls. When the semifrozen ice cream exits the freezer barrel, it expands as the pressure is lowered to atmospheric and when this expansion has been completed maximum overrun is achieved.

Continuous freezers enable production of ice cream of high overrun and low drawing temperatures. Air for overrun of up to 130% at draw temperatures of $-7.2^{\circ}C$ ($19^{\circ}F$) can be achieved with cylinder pressures of 3.5–5.5 atm (50–80 psig) depending upon the dasher and blade design and the condition of the blades. For overrun in excess of 130%, cylinder pressures may have to be increased further. When draw temperatures is lower than $-7^{\circ}C$ ($19^{\circ}F$) cylinder pressures may have to be increased by 2–3 atm.

The temperature of the mix entering the freezer is very important to freezer performance. If the temperature of the mix is uniform throughout the run, the overrun control and freezing rate are predictable, provided that the refrigerant supply and suction conditions are uniform. Temperatures of $0^{\circ}C$ ($32^{\circ}F$) will optimize freezer performance. However, to achieve such a low temperature of the mix a scraped surface heat exchanger may have to be used. Normal pasteurized mix temperatures are around $3–4^{\circ}C$ ($40^{\circ}F$). Newer freezer designs make it possible to extrude ice cream at $-18^{\circ}C$ ($0^{\circ}F$) creating some interesting and desirable texture characteristics.

The consistency of ice cream as it is drawn from the freezer is often referred to as "wet," "dry," or "stiff." The terms dry and stiff are used interchangeably in parts of the world. This consistency is influenced more by formulation than by any other factor. Mix that produces a characteristic wet ice cream can be reformulated to produce a dry product. Stiffer drier ice cream is advantageous when manufacturing novelties where the ice cream is manipulated to form different shapes. Flowable wet ice cream is preferred when filling containers of various sizes because such a product results in a uniform fill with no empty pockets. Stiff, dry ice cream when filled in containers can leave voids that consumers interpret as companies cheating them.

The capacity of continuous freezers is difficult to rate since frozen desserts have differing characteristics which in turn affect refrigeration requirements. There is no generally adopted standard among equipment manufacturers for rating freezer throughput. However, if the machines are new or in excellent operating conditions, refrigerants are oil free, the ice cream mix is approximately 10% fat, 15–16% sugar, 37–38% total solids and the mix enters the freezer at $4^{\circ}C$ ($40^{\circ}F$) and exits at $-5^{\circ}C$ ($23^{\circ}F$) and the refrigerant evaporating temperature is $-5^{\circ}C$ ($23^{\circ}F$) or 2 psig pressure a valid comparison can be made for ice cream throughput at 100% overrun. It is critical to have all conditions

illustrated above to be the same to make valid comparisons between manufacturers of equipment. The rating is a nominal value and it is a given that the ice cream manufacturer will rarely approach these ratings in day to day production.

74.4.6.5 Hardening. The aim of freezing ice cream is to convert approximately 50% of the water in the mix to ice. This is done by rapid freezing in the continuous freezer which also results in small ice crystals. The remainder of the water in the mix is frozen on to these newly created ice crystals as rapidly as possible in an operation called hardening. In order to harden ice cream the package of ice cream is placed in a very cold environment where large volumes of very cold air sweep the surfaces of the packages for a period of time. In such instances freezing of the remaining water on the already existing nuclei takes place from the outside towards the center of the package. As more water gets converted to ice it acts as an insulator. Therefore it takes considerable amount of time for the center of the package to reach $-18^{\circ}C$ ($0^{\circ}F$). It is recommended that the center temperature of a rectangular half gallon of ice cream reach $-18^{\circ}C$ ($0^{\circ}F$) in 3 h or less. In order to achieve the air temperature has to be at least $-28.9^{\circ}C$ to $-34.4^{\circ}C$ (-20 to $-30^{\circ}F$). A larger package, such a as 11.25 L (3 U.S. gallon) tub, will take a longer period of time to reach a center temperature of $-18^{\circ}C$ ($-0^{\circ}F$). Ideally a core temperature of $-18^{\circ}C$ ($0^{\circ}F$) should be reached in 9–10 h. Hardening rooms can batch or continuous. Batch hardening rooms consist of a very cold room with the ability to move large volumes of air about in this room. The products should arrange in such a manner that this cold air is able to sweep all surfaces of the packaged ice cream. This requires air spaces all around the cartons of ice cream. In a continuous hardening system the ice cream package traverses a box in which cold air is circulated. The ice cream enters this box on a conveyor belt at one end of the box and is conveyed in a repeating zigzag manner back and forth until the required residence time is attained. Then the hardened ice cream exits from this hardener and can be stored. Continuous hardening systems are called hardening tunnels.

Hardening apparatus configurations can be a room, tunnels, spiral tunnels, straight through tunnels, contact plate freezers, and special tunnels used in novelty manufacture.

In all hardening systems frost builds up in the room over time. In humid environments, this process may be quicker than in arid environments. The room or tunnel has to be defrosted. Avoiding or minimizing frost build up is desirable. It is important to keep the evaporators and other parts of the room or tunnel frost free. Defrosting entails raising the temperature enough to melt and remove the frost. It consumes energy to defrost and additional energy is required to cool the room or tunnel back down to operating temperatures.

Hardened ice cream is stored at $-28^{\circ}C$ ($-20^{\circ}F$) prior to distribution. The time to harden is affected by the package size and geometry, air temperature, air velocity and turbulence, package surface exposure to cold air and over wrapping, bundling, and so on.

74.5 PACKAGING

A good package must contain the product, protect it, provide convenience and provide information on the product to the consumer. Food packages provide protection against physical, chemical and biological damage. It also provides information useful to the consumer, for example, ingredient label, nutritional label, net contents, serving suggestion, and methods of preparing the product. Besides these attributes, a good food package

keeps the food at nearly the same quality as when it was manufactured. During distribution packages are subjected to physical abuses such as shocks, vibrations, compression, and in the case of ice cream and frozen desserts, heat shock.

For frozen dessert packaging three main factors have to be considered. First, the package has to protect against temperature fluctuations, photooxidation, dehydration, and odor transmittance. Second, it has to take into consideration distribution related factors such as package integrity, thermal shock and cube efficiency. Third, municipal solid waste management factors have also to be considered.

In a consumer study performed prior to the passing of the Nutritional Labeling and Education Act NLEA, 90% of the consumers polled wanted tamper evident packaging and nutritional information (particularly calories and sodium contents). The latter is now mandated in a defined format by NLEA and has adequately addressed that concern. Almost 80% of the consumers desired ice cream packages that hold up better and not get soggy. Also noted were resealable rectangles and cartons that shrank as product was used.

Ice cream was packaged in the 1920s in paperboard tapered pails with handles with easy open tabs and was of small size for total consumption. In the 1930s various configurations of packages which were small enough to fit into the ice cube tray compartments of home refrigerators paperboard containers appeared. Such containers were no larger than a quart and were reclosable tab lids. In the 1940s and 1950s the package size increased to half gallon with appropriate strength to be filled automatically and had reclosable tabs at either end. In this era round containers were positioned as premium products. In the 1960s through 1970s plastic rounds, two piece cartons coated for higher quality graphics, zippered tab opening and hooded hinged lids were introduced. In the decades of 1980s through 1990s tamper evident shrink film and bands, two place containers in a wide range of materials, transparent and translucent packages, membranes inside packages to reseal, and rectangular packages with rounded corners were some of the notable developments. These evolutionary patterns follow the penetration of domestic refrigerators and the changes in sociological and demographic factors.

Regardless of the container shape and material of construction, ice cream packages are often shrinkwrapped and then sleeved in singly or in pairs prior to entering the hardening systems. The shrink wrap is an indication of tampering but it also provides an additional layer of protection. It is a two-edged sword in the sense that in addition to providing an extra layer of protection the heat applied to seal may cause heat shock and more importantly, reduce heat transfer rates during the hardening phase of manufacture. This can result in longer times for hardening ice cream and act as a capacity constraint.

As far as protecting the product from heat shock goes, there a number of variables which have an impact on this. The cold chain which consists of the steps involved in moving ice cream from the factory to the consumer is a major variable. The number of steps involved may be as simple as moving the ice cream from the hardening room to point of sale as may be the case in a small shop to as complex as moving ice cream from the factory to a factory warehouse, then to retailer's distribution center and from there to the store freezer, display freezer, transport home, and finally consumption. Transportation is involved in this chain and distance and altitude of travel may also play a role. Because of these variables it is often hard to arrive at a reliable sell-by date for ice cream. The problems may be exacerbated in the case of novelties because generally these products are smaller in volume and therefore more susceptible to melting and refreezing. In many instances manufacturers do not know the extent of temperature variation in their distribution system.

TABLE 74.8 Effects of Packaging Materials, Display Cabinet Style, and Temperature of Storage on Shelf-life of Ice Cream.

Package Material	Time Until Product is Not Fit for Sale (Weeks)			
	Vertical Cabinet			*Open display*
	−12°C	−15°C	−18°C	−15°C
	Cartonboard Packages			
Hot melt coated	8–10	11–16	22	Nd
PE-coated	6–8	11–16	13–17	10
Aluminum foil laminated	Nd	Nd	Nd	16–18
	Plastic Packages			
PS-box	16	16–22	>30	Nd
HDPE-box	16	19	22–26	Nd

Abbreviations: PE = polyethylene; PS = polystyrene; HDPE = high density polyethylene; Nd = not determined.

In a Finnish study conducted in 1984 compared different package types stored at different temperatures in both chest and upright freezer cabinets. Product acceptability was evaluated using a sensory panel (Table 74.8).

74.5.1 Storage and Distribution

Frozen and hardened product is stored and often distributed prior to the enjoyment by the end consumer. The intermediate steps involved in storage vary depending upon scale of manufacture, market share, point of sale, and consumer preferences. In the simplest case of a retail ice cream manufacturer, the product is made fresh in the store and sold very soon after manufacture and this requires relatively few controls. In an extreme case, the product made on one of the coasts of the United States is transported for sale to the opposite coast. Here, time, transport conditions, altitude, temperature, humidity, refrigeration conditions, and so on, have to be carefully controlled in a manner that the frozen dessert maintains its quality when the consumer eats the product.

Ice cream is unique in that it is the only product that is consumed in the frozen state. Therefore, once it is manufactured it has to be stored, transported, distributed, and sold in the frozen state. In the United States, frozen foods are distributed in a separate chain than ice cream is because the cold chain for frozen foods is −18°C (0°F) and is inadequate for ice cream. Ice cream cold chain maintains −23°C (−10°F). The distribution chain is called the cold chain and varies from manufacturer to manufacturer. Regardless of the variations one thing is certain. The cold chain is imperfect. This imperfection affects the quality of the product at the point of purchase and impacts consumer satisfaction. Factors affecting the shelf-life of ice cream are manufacturing procedures, warehouse equipment, warehouse handling practices, transportation, storage at retail premises, retail display equipment, and retail handling practices.

74.5.1.1 Manufacturing Procedures. Mix formulation, the adequacy, functionality, and quality of ingredients are all important parameters that receive careful attention. This is followed by proper blending to achieve intended functionalities of the added ingredients. Next pasteurization and homogenization, freezing, packaging, and hardening

have also to be carefully monitored and controlled. Packaging and outer case for products should be of good quality to prevent contamination, ensure integrity of product during normal storage and transportation, minimize dehydration and also but not the least package coding should be adequate for effective identification. Outer coding is useful for proper stock rotation and phrases such as "store at $-29^{\circ}C$ ($-20^{\circ}F$) or colder" should appear on outer cases. Lot pallet or unit load identity is useful in proper stock rotation while maintaining lot identity.

74.5.1.2 Warehouse Equipment. Warehouse should be of adequate capacity, suitably refrigerated to maintain a steady air temperature of $-29^{\circ}C$ under peak loading conditions and maximum ambient temperature. Storage areas must be equipped with accurate temperature recording devices. Daily checks of temperatures of each area in the warehouse should be maintained and recorded. Automated recorders are preferable and data should be retained for a 2-year period. Warehouse operator should record the product temperature of each lot of product received and should accept custody only in accordance with good commercial practice. Records for arrival lot temperatures should be retained for a period of 1 year.

If products are received above $-29^{\circ}C$ the operator should immediately notify the owner or consignee and request instructions for special handling. Product received above $-29^{\circ}C$ had incurred heat shock and may pose quality problems. Before placing the shipment in storage it should be code marked for effective identification. Every effort should be made to minimize exposure to elevated temperatures and humidity conditions.

During defrosting operations cover products beneath areas of accumulated frost. Products going in to the staging areas for order assembly must be moved out of the area promptly unless the staging area is maintained at $-29^{\circ}C$. As many of the operations should be carried out in the cold if possible. Allow the bottom of the stack to have air passage and leave adequate room between stacks for proper air circulation.

74.5.1.3 Transportation. Vehicles used for transporting ice cream products should be clean, free of dirt, offensive odors, debris, and so on They should also be insulated and equipped with adequate refrigeration. The vehicle must have tight-fitting doors without air leaks and should be precooled for 25 min prior to loading. Transport vehicles must also have accurate, visible, and readable temperature recording devices. The thermostat on the truck should be set to maintain an air temperature of $-29^{\circ}C$ with proper airflow or circulation. During loading and unloading operations the refrigeration unit must be off.

74.5.1.4 Storage on Retail Premises. Storage on retail premises must have adequate refrigeration capacity to maintain a product temperature of $-29^{\circ}C$ and of sufficient size to maintain proper stock control and rotation. Storage facility must have adequate circulation of cold air all around the products. Storage facilities must be equipped with an accurate, readable temperature recording device which is easy to calibrate. Facility should be defrosted regularly as necessary to maintain refrigeration efficiency.

74.5.1.5 Retail Display Equipment. Display cabinets should be capable of maintaining $-29^{\circ}C$ and should be situated away from drafts, direct sun, heat producing equipment, or any other factor likely to reduce its efficiency. The display case should have a calibrated easy to read thermometer at a location representative of the average temperature

of the cabinet. Display cabinets should have a properly marked load limit on the cabinet walls. To facilitate air circulation cabinets should have sufficient dividers, separators, and grids. Cabinets must be defrosted when necessary to assure proper operation. It should be kept free of debris, signs, and tags which deflect refrigerated air flow.

74.5.1.6 Retail Handling Practices. Ice cream and frozen novelties should be delivered in a frozen condition at $-29^{\circ}C$. Warmer products should be rejected or if accepted examined for quality and put up for quick sale. Once unloaded at the retail end, the products must be moved quickly to the freezer. Inventory must be rotated on a first-in, first-out basis. Any cases not bearing a code or date should be dated upon receipt. When loading ice cream into display cabinets rotate inventory already in the cabinet. New products should be placed beneath the existing stock. Items should not be placed outside of the designated load limit lines and care should be taken not to block air flow. Store personnel should be aware of maintenance and sanitary upkeep of the freezers.

For the operation of efficient and optimal cold chain educational programs for all personnel handling ice cream must be implemented. This will insure that the customers receive products in the best possible condition.

74.5.1.7 Heat Shock. Heat shock is a term used to describe temperature fluctuations that occur during the storage and distribution of ice cream. The aim is to minimize heat shock and its deleterious effects. Rise and fall of temperatures affect the water–ice equilibrium in frozen desserts. During the freezing and hardening of ice cream extreme care is taken to create the largest number of small ice crystals. Ice crystal nucleation is initiated in the ice cream freezer where the objective is to freeze approximately 50% of the water in the formulation as quickly as possible. The remainder of water that can be frozen changes state in the hardening process. No new ice nuclei are created in the hardening process. Following the laws of thermodynamics large ice crystals grow at the expense of small ice crystals. Every time the temperature rises some of the ice melts and when the temperature goes down it refreezes. The refreezing takes place on existing ice nuclei and thus the number of small ice crystals decreases while that of larger ice crystals increases. The amount of ice thawing and water refreezing is dependent on the extent of temperature fluctuation and the frequency of this fluctuation. When ice crystal sizes reach 150 μm the coarseness begins to get apparent to the tongue. Other effects of heat shock are the loss of shape and eye appeal of novelties and if persistent loss of market share for the manufacturer.

Depending upon the solute composition of the ice cream mix a freezing and thawing curve for that mix can be constructed (Fig. 74.5). Such a curve can provide the amount of ice and water changing state for defined temperature change. At very low temperatures relatively little water/ice changes state.

As the temperature warms more, water/ice change state. If a greater amount of water/ice changes state the shelf life of the product will shorten considerably when compared to a much smaller change in temperature. An example of the field data of temperature changes during storage and distribution of ice cream is provided (Fig. 74.6).

In this illustration the ice cream was “all natural” with no added stabilizers or emulsifiers. The overrun was approximately 100%. Whenever the product was in transit the temperature fluctuations were large. When the warehouse was under the management of the manufacturer product handling was good. Whenever warehouse control was

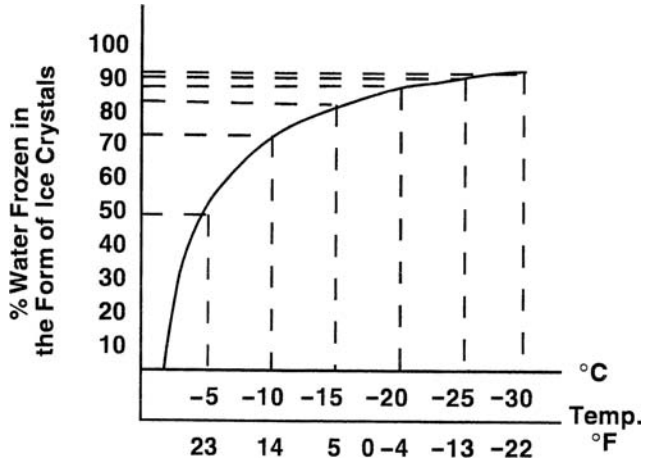

Figure 74.5 *Freezing / thawing curve for a typical ice cream mix.*

relinquished by the manufacturer the temperature fluctuations increased. In this example the product was coarse within 90 days.

Display cabinets at the retail point of sale are also notorious for causing heat shock to the product. The common types of display cabinets are open top merchandisers (also known as coffin style) and upright with glass doors. Consumers also keep doors open while making their choices leading to frost build up and necessitating frequent defrost cycles, which in turns adds heat shock. A survey of ice cream manufacturers in the United States revealed that physical defects were more frequent than flavor defects.

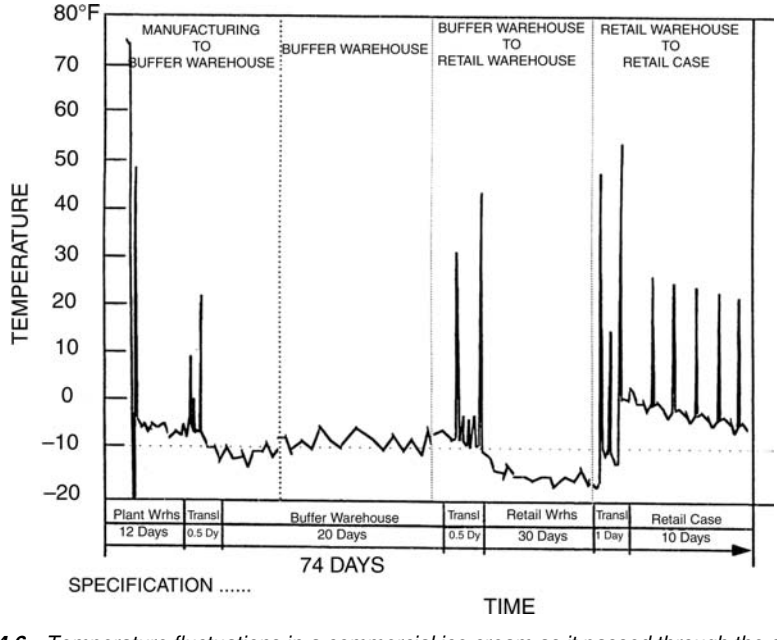

Figure 74.6 *Temperature fluctuations in a commercial ice cream as it passed through the cold chain.*

Customers reacted unfavorably to physical defects. Defects occurring often were reported to be icy texture, shrinkage, gummy/sticky texture, and evidence of previous melt. Customers were more tolerant of icy or coarseness than other physical defects. These defects objectionable to the consumers are all a result of heat shock.

If rectangular 1.88 L (U.S. 0.5 gallon) containers of ice cream are placed in an open top merchandiser packed three deep and the merchandiser undergoes two defrost cycles per 24 h the temperature fluctuations are as depicted in Figure 74.7.

As expected, the carton on top undergoes the greatest fluctuation and the cartons on the bottom of the pile undergo the least fluctuations. In such cartons the product contained in the outside 2 cm represents 60% of the total ice cream in the container while the center 2 cm portion represents only about 15% of the volume of the container. In approximately 4 days the amount of water that is formed and converted to ice is equivalent to the total water that was originally present in the mix.

74.6 NUTRITIONAL VALUE OF ICE CREAM

Table 74.9 gives an example of nutritional label of ice cream. It is evident that ice cream furnishes vital constituents and nutrients of milk, such as protein, calcium and other minerals, as well as vitamins.

74.7 MANUFACTURE OF ICE CREAM NOVELTIES

Novelties are products that have unique characteristics such as shape, color, and packaging. Typically novelties offer convenience, portion control (individual serving), unique forms (shape, size, color, flavor, and package). These are value added products conducive to impulse purchase. Novelties can be classified as sticks, bars, slabs, bite size, cups, and cones. These products can be molded that is, shaped by pouring and freezing mix in a mold or extruded. The products can be ice cream, water ice, sherbet, or a combination of these.

74.7.1 Molded Novelties

Novelties that acquire their shape from a mold or form are called molded novelty items. In such products a mold is filled with a mix or ice cream and then frozen further. The product is then demolded (removed from the mold) and enrobed in or coated. Most often molded products are stick products. Sticks are inserted into the semifrozen product in a mold and

TABLE 74.9 Comparisons Between Molded and Extrusion Processes.

Characteristic	Molded	Extruded
Ice cream temperature	−3 to −4°C	−6 to −7°C
% Water frozen	25–35	55–60
Texture from freezer	Fluid	Stiff
Flow	Intermittent	Continuous
Shape defined by	Mold	Orifice
Texture after hardening	Coarse	Smooth

then the freezing process is completed. When such a product is demolded the stick serves as a means of holding the product while eating. Equipment that manufactures molded novelties can be straight line or rotary machines. In a straight line machine there is a linear array of molds. Molds can be easily changed. Straight line machines occupy more floor space, are easier to clean and sanitize but the water use is greater as is the waste generation. In rotary machines molds are arranged radially on a large circular wheel and this configuration uses less floor space than straight line machines. There is less product lost in the molds but cleaning is more difficult and mold changes are time consuming. Molds are made of special alloys that have good heat transfer capacity, mechanical durability, resistance to brine, and resistant to corrosion from cleaning and sanitizing solutions. Molds can also be made from plastics which have particular advantages in three-dimensional effects. Generally molds are 0.22 mm (0.75 in) thick and volumes of molds vary from 50 mL (1.8 fl oz) to 75 mL (2.8 fl oz). Molds are held in place several molds wide on a rotating conveyor belt. In a straight line machine the molds traverse from one end of a cold brine trough to the other. Various operations are conducted in stages during this travel from end to end. There are also rotary machines in which the molds rotate around a drum containing cold brine and various operations can be staged at different points in this rotation. In either case the novelty making process starts with charging the molds with ice cream and ends with the demolded product transferred to packaging stations.

The steps in molded novelty manufacture involve filling the mold with partially frozen mix. The filled molds are frozen to a consistency that can support a stick upright and then the stick is inserted. Freezing proceeds further until a stiff consistency is obtained. Then the product is demolded. Demolding takes place by passing the molds through a warm brine zone where the outer layer of the product is softened enough so that the product can be removed from the mold. The product is removed from the mold by lifting it from the mold. The removed product can then be dipped in water, chocolate coating, and so on. The coated products can then be dipped in nuts, candy, sprinkles, and so on, prior to packaging. Some coatings may require a refreezing of the product. This refreezing can be accomplished by immersion in liquid nitrogen. This step may be repeated several times to build up a thick layer of the coating. The packaged product are put into multipacks or cartons and sent for hardening. The terms used in molded novelty manufacture are as follows: "wide" refers to the number of individual molds in a row of the machine, for example, eight wide means eight molds in one row or 12 wide means 12 molds in a row. "Strokes" means number of pieces produced in one synchronous movement. It is a measurement of speed. Typical strokes are 16–24 per min. So an eight wide machine operating at 16 strokes per min produces 128 pieces per min or 7680 pieces per h.

Several variations can be made in the manufacturing process. For example, one-third of the mold can be filled with one flavor, partially frozen, and a second flavor added to make two-thirds of the volume, and finally another third of a different flavor can be layered on top prior to stick insertion. A second instance of variation is what is called shell and core freezing. In this type of stick novelty the outside has a different flavor and product than the inside. This is achieved by filling the mold with one flavor and allowing that flavor to freeze along the inner edges of the mold; the unfrozen mix is aspirated from the mold followed by the filling of a second flavor or type of product. Freezing, sticking, demolding, and packaging then continues after these stages. By controlling the extent of product frozen in the mold prior to aspiration of the unfrozen mix different thicknesses of flavors can be achieved.

74.7.2 Extruded Novelties

In the manufacture of this type of novelty ice is extruded through an orifice and then separated into individual portions (frequently by a heated wire). Extruded novelties can be stick or stickless products. Sticks are inserted immediately after extrusion and prior to cutting. Extrusion can be horizontal or vertical. Horizontal extrusion is also called band extrusion. Coextrusion is also possible where more than one type of ice cream is extruded through the orifice. Cups, cones, and other filled products can be made by extrusion. Extruded portions are then hardened in a spiral freezer. Since the volume of the extrudates is small hardening can be achieved rapidly. Hardened products can then be coated/enrobed and packaged. The types of extruded novelties include cups, cones, sandwiches, cakes, bite size miniatures (e.g., bon bons), and candy bars style products. Extruded items can be decorated during extrusion. A small orifice in the shape of a star is often used to create a rosette and up to eight such devices can be used simultaneously to decorate an ice cream cake. The true expertise in the manufacture of molded novelties is in the design of the extrusion nozzles and in conceiving unique shapes or forms. Comparisons of molded and extruded novelties are described (Table 74.10).

This chapter has provided an overview of the manufacture of ice cream and other frozen desserts. There obviously are many more facets to this complex process. Changes occurring at a molecular level, the physicochemical basis for structure formation, sensory evaluation methods, proper cleaning, and sanitation procedures and new developing trends in the industry are not discussed in this article. Their omission here must not be misconstrued as the lack of importance of these topics. Additionally, the demand for ice cream and frozen desserts is increasing globally. With this global demand newer flavors, nutritional concerns, and cost-effective manufacture and delivery of these tasty treats gains greater importance.

TABLE 74.10 Nutritive Value of One Serving ($\frac{1}{2}$ cup, 4.4 fl oz, 120 mL, or Approximately 66 g) of Ice Cream.

	Vanilla Ice Cream, 4% Fat	% Daily Value*	Vanilla Ice Cream, 10% Fat	% Daily Value*	Vanilla Ice Cream, 16% Fat	% Daily Value*	Orange Sherbet, 2% Fat	% Daily Value*
Total calories, Kcal	123.4		133.6		174.7		135.8	
Calories from fat, Kcal	34.1		64.0		106.6		17.3	
Total fat, g	3.8	6	7.1	11	11.8	18	1.9	3
Saturated fat, g	2.4	12	4.4	22	7.4	37	1.2	6
Cholesterol, mg	12.2	4	29.5	10	43.8	15	7.1	2
Sodium, mg	70.2	3	57.6	2	54.1	2	44.4	2
Total carbohydrate, g	19.5	6	15.7	5	16	5	29.5	10
Dietary fiber, g	0	0	0	0	0	0	0	0
Sugars, g	18.4		14.8		15.1		27.8	
Protein, g	3.5	7	2.4	5	2.1	4	1.1	2
Vitamin A (IU)	143.4	2	269.3	6	448.4	8	93.1	0
Vitamin C, mg	0.5	0	0.4	0	0.3	0	1.9	4
Calcium, mg	118.3	10	87.2	8	75.6	8	52	6
Iron, mg	0.1	0	0.1	0	0.1	0	0.2	0

*% Daily value is based on 2000 calorie diet.

Source: Nutritive Value of Foods, Home and Garden Bulletin Number 72, October 2002. Available at www.nal. usda.gov/fnci/foodcomp.

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75

Frozen Novelties

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75.1 Introduction 635
75.2 Quiescently Frozen Products 636
75.3 Stirred Novelties 640
75.4 Extruded Novelties 641
75.5 The Changeable Nature of Novelties 644
References 644

75.1 INTRODUCTION

Fully, a quarter of the frozen dessert products sold in the United States are in the novelty category and this proportion has been growing (IDFA 2003). Vending machine sales of frozen novelties exceeded 8.5 million units in 2004 (IDFA 2005). The total retail value of the novelty category was 5.2 billion dollars in 2004, which accounted for 24% of the ice cream industry for that year (IDFA 2005). Supermarket sales of frozen novelties accounted for 2.3 billion dollars in 2004 (IDFA 2005). The highest sales, by volume share in 2000, were frozen ices (26%), ice cream bars (19%), ice cream sandwiches (13%), and fruit juice bars (9%) (IDFA 2001).

Items made by freezing a mix of flavor and other ingredients with water were developed much earlier than ice cream. It is likely that a sweet food froze serendipitously at some point in the past (perhaps even with an embedded stick), and someone found that they liked the result. Mankind tends to take advantage of chance occurrences like this. Perhaps initially artisans and chefs made these products for their affluent customers. It has been reported that the emperor Nero imported ice form the mountains to be combined

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with fruit juices in the 4th century BC (Bellis 2005). In recent times, after the development of refrigeration, mass production of novelty products could be distributed to more and more people. Today there are vast numbers of these products sold in huge quantities in virtually every country on earth.

On a basic level, this enormous variety of different frozen novelties can be broken into three groups. Novelties that are frozen by placing the fluid ingredients into a mold and then freezing that product without further stirring are classified as quiescently frozen. An example of this kind of product is the conventional ice pop or ice-lolly. Products that are placed into a mold after being partially frozen in a conventional ice cream freezer are classified as stirred products. Ice cream frozen to a slurry consistency, deposited into a mold to finish freezing, removed from the mold and coated with chocolate is an example of this genus. The third group comprises those products that are frozen in a conventional ice cream freezer, extruded and cut to form the shape of the final product. These are called extruded novelties. Perhaps the most common example of this type of novelty is the ice cream sandwich. Extruded novelties can be much more complicated than this. They include products like ice cream on a stick shaped like a cartoon character with colored ice cream embedded in the extrusion to represent the various parts of the character.

75.2 QUIESCENTLY FROZEN PRODUCTS

Quiescently frozen novelties can either be simple or complex. They may have a single flavor/color or many colors and flavors layered, swirled, or otherwise cast within the product. Since the shape of the novelty is determined by mold geometry (Fig. 75.1), the number of possible shapes is infinite, though shapes that lend themselves to easy removal from the molds are most common. Perhaps the simplest novelty in this genus is the ice pop. This product is basically a frozen solution of water, sugar, flavor, and color, mounted on a stick.

The two most common quiescently frozen products are ice pops and fudge bars. An ice pops is basically a frozen syrup containing sugars, flavor, and color. The ingredients in ice pops can be partially substituted for by fruit juice, to make juice pops. Similar to the ice pop, but slightly more complex, is the fudge bar. Fudge bars often include some fat and have a higher solids content than ice pops. Fudge bars and similar products

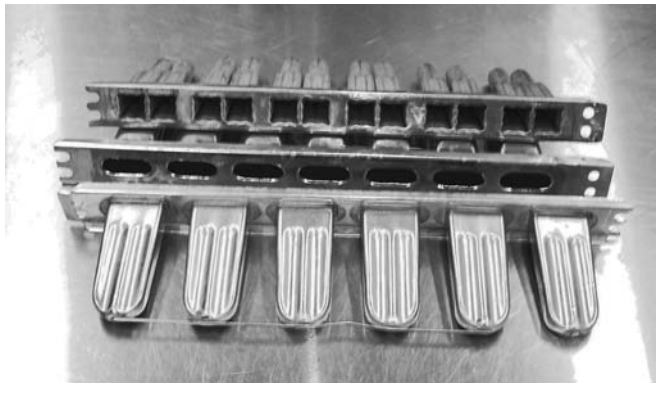

Figure 75.1 *Various molds for quiescently frozen novelties.*

TABLE 75.1 Formulations for Quiescently Frozen Products.

Component	Ice Pop (%)	Sugar Free Ice Pop (%)	Fudge Bar (%)	Nonfat Fudge Bar (%)
Milk fat	—	—	3.0	—
Milk solids nonfat	—	—	9.5	8.5
Sucrose	15.0	—	13.0	13.0
36DE corn syrup solids	4.0	—	2.0	4.0
Citric acid	0.8	—	—	—
Cocoa	—	—	2.8	3.0
Polydextrose	—	8.0	—	—
Sorbatol	—	8.0	—	—
Artificial sweetener	—	0.01	—	—
Stabilizer	0.4	0.45	0.3	0.3

have a smoother texture than ice pops, attributable to smaller ice crystals and a higher content of unfrozen solution at consumption temperature that result from formulations that contain higher solids and fat. Pudding bars have similar formulations, without cocoa. Table 75.1 contains some representative formulations for ice pops and fudge bars.

During the freezing process, the fluid mix is deposited into the mold. The mold is suspended in a temperature exchange medium. This medium can be a calcium chloride brine, glycol, forced air, liquid nitrogen, or any other safe medium that can carry away heat from the product as rapidly as possible. Each of the media has advantages and disadvantages and it is best to consider the situation and consult equipment suppliers as to which is best for a specific product. As the product is not stirred, the solution in contact with the sides of the mold will cool more quickly than it will near the center of the pop. This fact is critical to both the texture of the pop and to its manufacture. When the solution becomes cold enough, the water in the solution begins to crystallize. The actual temperature at which this happens is dependent on the formulation of the product. Dendritic (needle shaped) crystals begin to form on the surface of the mold. Ice crystals are formed of pure water (Korber and others 1992). Because of this, the other components in the mix are mechanically swept out ahead of the growing ice crystals. This results in a concentration gradient forming in the vicinity of the ice crystals. The number of crystals forming and the eventual size of the ice crystals in the product are affected by this concentration gradient. The concentration gradient in the mix forms, during freezing, with the highest concentration near the ice crystal and the lowest in the areas not yet containing ice crystals. The viscosity in the gradient near the growing crystals increases as a result of this concentration (Bronstien and others 1991). Also as a result of this physical process, the freezing point of the liquid close to the ice crystals is depressed. Because the freezing point of the solution in the gradient is low relative to the bulk solution, and because the viscosity of the mix near the growing crystal is relatively high, the formation of new ice on the existing crystals is impeded in this area.

Since heat continues to be removed by the cooling medium, there is a tendency for new ice crystals to form where the solution is less concentrated. The result is the generation of smaller ice crystals. The sugars and other small molecules in the mix control the effect on freezing point. The effect on viscosity is due to both the sugars and to the hydrocolloids used in the stabilizer. Solutes can more easily diffuse away from the forming ice crystals during slow freezing than during rapid freezing. The reason for this is that there is more time for diffusion to take place. Larger ice crystals are, therefore, the result of slow

freezing. Larger ice crystals are perceived as a more course texture that, depending on the product, may not be desirable. Equipment, processes, and formulations can be designed to produce a variety of textures by manipulating the rapidity of the freezing process.

During the freezing process, all the solutes are concentrated in the spaces between the ice crystals (Korber and others 1992). At some point (depending on formulation and rapidity of the freezing process) the unfrozen solution becomes so viscous that water is prevented from reaching the forming ice crystals. Because of this, additional ice cannot form. The ultimate concentration of the material surrounding the ice crystals depends on the temperature of the product and on the number of individual molecules present in a unit volume of the solution. With small ice crystals, this concentrated material is more uniformly distributed throughout the product than it would be if large crystals predominated. This difference in distribution can be detected in the mouth and is interpreted as smoothness/coarseness.

During freezing the general direction of ice crystal formation is towards the geometric center of the novelty (towards the center of the mold). At the point when the ice crystal front has nearly joined at the center, the stick is inserted. Stick inserting mechanisms are placed at this point in the freezing process line. The actual position is determined by eye (manual or mechanical). This allows the stick to remain vertical until the completion of freezing. The exclusion of solutes including flavoring and coloring from the growing ice crystals forces theses materials toward the center. In extreme cases the stick can become covered by a viscous layer of concentrated sugar, flavor, color, and stabilizer, resulting in potential customer complaints.

After the product has been completely frozen the apparatus advances the molds into a warm bath (the temperature and duration of this bath are determined by the equipment and the product formulation). At this time the mold is heated just enough to permit the removal of the bars. In the case of elaborate molds like spirals, the withdrawal procedures can be quite complex, involving twisting, mold disassembly or other operations. After withdrawal the bars are wrapped and boxed and sent to the distribution freezer.

In formulating novelties, and for that matter all frozen products, it is necessary to take into account the freezing point of the unfrozen mix. Too high a freezing point yields a product with a hard texture at serving temperature and too low a freezing point will yield a product that melts and drips at serving temperature. The freezing point of any solution is determined by the solutes. For frozen desserts these are primarily in the carbohydrate content of the mix. Salts and other small molecules like ethanol can also affect the freezing point and must also be accounted for in formulations that contain them. It is the number of molecules present that determines the degree of freezing point depression, not the type of molecule. Since there are more molecules in a unit weight of a small molecule like fructose, the freezing point will be depressed more than would be the case if the same weight of a larger molecule like sucrose were used. Likewise, huge molecules like hydrocolloids and protein, have little effect on the freezing point of mixtures that contain them. Table 75.2 contains a list of common carbohydrates found in frozen desserts, their molecular weight, and effect on freezing point as compared to sucrose. Figure 75.2 shows this relationship in general.

At storage temperature (below $-18^{\circ}C$) the product will contain ice crystals and an amount of unfrozen solution that depends primarily on the solute content. The unfrozen solution is very viscous, if not glassy, at storage and consumption temperatures. This viscosity, along with the type and size of the ice crystals imbedded in it, will contribute to the eating quality of the dessert. In addition to the simple carbohydrates in the product, the

TABLE 75.2 The Relationship Between Molecular Weight and Freezing Point Depression for Some Common Carbohydrates.

Carbohydrate	Average Molecular Weight (Daltons)	Change in Freezing Point Relative to Sucrose[a]
Sucrose	342	1.0
Fructose	180	1.9
Glucose	180	1.9
Lactose	342	1.0
Sorbitol	182	1.9
Lactitol	362	1
Xylitol	152	2.3
Maltitol	362	1.0
Erythritol	122	0.9
Glycerin	99	3.7
Polydextrose	>22,000	Negligible
High fructose corn syrup (42%)	190	1.8
36DE corn syrup solids	472	0.7

[a]Factor used to calculate the reduction in freezing point on an equal weight basis as compared to sucrose. For example, the reduction in freezing point for a given weight of fructose is nearly twice (1.9) as much as the reduction for the same weight as sucrose.

hydrocolloid content affects the texture of the products. The hydrocolloid content primarily affects the viscosity and gel like nature of the concentrated solution. Manipulating the hydrocolloid type and content can achieve many variations in texture. For example a slimy texture for children's novelties can be achieved using xanthan gum. The addition of modified food starch can give a pudding-like texture. Even gelled textures, where the dessert does not drip but becomes a gel upon melting, are possible. There is no ideal texture for novelties; often a defective quality in one novelty is the desired result in another.

While the desired texture and flavor of novelties may require a low freezing point, this low freezing point can make novelty products susceptible to damage at storage temperatures and temperatures during distribution. At storage and distribution temperatures producers want products to be as hard as possible, in order to survive distribution. In order to make products more resistant to damage a coating of frozen water or fat is often

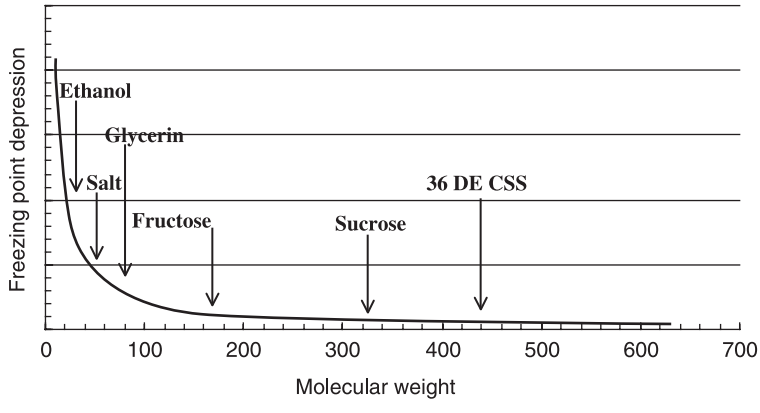

Figure 75.2 *The relationship between molecular weight (Daltons) and freezing point depression relative to sucrose on an equal weight loss.*

applied to the novelty (Frank 2000). This gives a more rigid exterior to the novelty that helps maintain shape when temperatures are above or near the melting point of the interior. A layer of frozen water coating the bar also helps prevent the paper wrapper from adhering to the bar during storage.

Including fat contributes to the body and texture of quiescently frozen products. Fat provides points of discontinuity within the structure of the product. Fat obstructs the formation of long needle-shaped crystals within the product. Further, the advancing ice front is slowed or blocked by large solid particles like fat. The result of the presence of fat or other colloids is a smaller ice crystal average size and therefore a smoother texture. Fat also coats the mouth and lubricates during mastication, further altering the perceived texture of the product. This lubricity is largely a function of the melting profile of the fat. In cases where fat is desired in the formulation, the melting profile of the fat should be considered. Fats with a high melting point will not melt as quickly in the mouth in a frozen product as they might in a product served at room temperature. Sometimes this can be perceived as a sandy or gritty texture.

75.3 STIRRED NOVELTIES

In the stirred novelties group of molded novelties, the mix is partially frozen in an ice cream freezer prior to depositing it into molds. Within the freezer the product begins to form ice crystals and air is whipped in (overrun), though the product is still quite fluid in texture. On reaching the desired temperature (-2 to -3°C depending on the product formulation) this partially frozen aerated slurry is then pumped into a hopper, deposited into molds, and frozen quiescently. The final texture of stirred novelties is much creamier and smoother than those that are simply frozen quiescently. The presence of air, partially agglomerated fat and more numerous ice nucleation sites produced in the ice cream freezer results in smaller, less continuous ice crystals forming during the quiescent freezing step. This results in a smoother, creamier texture in the final product. Recently, filling technology has been introduced where high viscosity (frozen to a lower temperature -5 to -6°C) ice cream and similar products are introduced under pressure to the bottom of the mold (bottom filling) (Hansen 2004). This allows a much higher quality to be achieved in stirred novelties. Because the ice cream in bottom filled novelties is more viscous, the product is able to suspend inclusions like fruit and nuts (Hansen 2004), This makes possible many more products.

Stirred novelties are often coated with chocolate and sold as ice cream on a stick. There are many coatings that can be applied to the outside of the bars after they have been removed from the molds. Chocolate is one of the most prevalent coatings. Although pure "chocolate" can be used as a coating it is often not appropriate for frozen novelties. Pure chocolate contains cocoa fat that is very hard and brittle at low temperatures. A softer vegetable oil is often more suitable for these applications (Marshall and Arbuckle 1996). The fat content (55–70%) of coatings is often higher than for pure chocolate (Frank 2000). Lecithin is added to coating compounds to prevent thickening of the mixture due to moisture build-up during prolonged dipping (Turnbow and others 1992). The uniformity of the coating and its thickness after dipping is a function of the viscosity of the coating. Careful control of the composition and temperature of the coating mixture is critical to this control. The temperature of the dipping mixture is dependent on the composition of the coating and the desired thickness. It is important that coated bars be removed to a freezer quickly after dipping to remove the heat absorbed during dipping. Table 75.3 contains some examples of chocolate coating formulations.

TABLE 75.3 Chocolate Coating Formulations.

Ingredients, %	Dark	"Milk" 32% Fat
Fat	26–34%	28–34%
Cocoa powder	15%	6%
Skim milk powder	6%	15%
Sugar	45–53%	45–51%
Lecithin	0.4%	0.4%
Vanilla flavoring	0.2%	0.2%

Source: Danisco (2003).

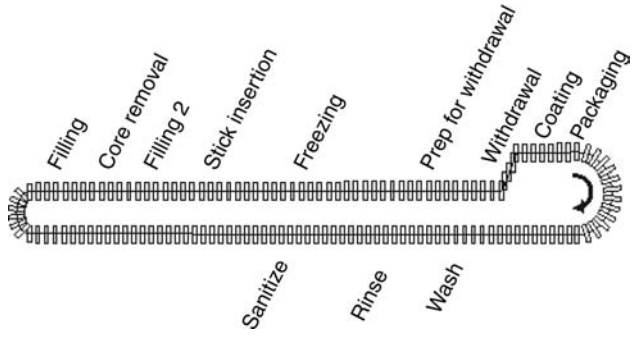

Figure 75.3 *Diagram of a typical quiescently frozen novelty process showing positions for mold filling, stick insertion, withdrawal, packaging and sanitation.*

Another stirred novelty product is made by first adding a fruit flavored sherbet or sorbet mix to the molds and allowing this to subsequently become partially frozen. The unfrozen core of the fruit mixture is removed by suction. In most equipment designed for this kind of product, the position (time) at which the core is removed is adjustable. This position is set usually by eye or by weight of the remaining shell. Ice cream is deposited into this shell and the freezing process is completed. The result is a bar with a fruit flavored shell on the outside and ice cream on the inside. A schematic for this process is shown in Figure 75.3. Close control of viscosity, temperature, and mix composition for all the components of these novelties must be made in order to assure uniform and predictable results.

By combining shells, fillings and coatings, a great many novel products can be created. Novelty equipment suppliers have designed a numerous different molds and equipment for extracting the novelties from the molds, which has enabled seemingly impossibly-shaped novelties to be easily formed. Interesting examples include spirals and animal forms in a rainbow of colors and flavors.

75.4 EXTRUDED NOVELTIES

Another basic group of novelties are extruded products. These can include shaped novelties, ice cream sandwiches and enrobed ice cream bars. Ice cream, sherbet, sorbet, and water ices can all be extruded. Extruded products are produced by first freezing a mix in an ice cream freezer to about -6°C. The product is then passed through a shaped nozzle or orifice. The product is then cut into slices using a blade or electrically heated

Figure 75.4 *Shapes possible using extrusion techniques.*

wire cutter (Marshall and Arbuckle 1996). If necessary, a stick is inserted and the product is hardened in a blast freezer (−30 to −40°C). Enrobing and decorating can occur after the product has been completely cooled to at least −15°C (Marshall and Arbuckle 1996). Equipment has been designed to produce virtually any shape and pattern. Some potential examples are shown in Figure 75.4.

One of the simplest novelties made in this manner is the ice cream sandwich, which consists of two flat cookies with a layer of ice cream between them. The ice cream is continuously extruded in a ribbon sized to fit width of the cookies and the desired thickness of the sandwich. Simultaneously, the cookies are placed on either side of the ice cream ribbon by the filling machine. The mechanism advances and the trailing cookie cuts off the ribbon of ice cream. (In some cases a wire cutter is employed in this step.) This advance sets the mechanism for the formation of the next sandwich. The sandwich then advances to a mechanical wrapper. The equipment is timed to deliver the correct amount of ice cream between the cookies without any undesired overhang. After wrapping, the ice cream sandwiches are boxed, palletized, and blast frozen. This whole process is a complex and precisely timed operation with the ice cream ribbon being extruded, the cookies being inserted the sandwich being formed, advanced (cutting the ice cream ribbon), and wrapped in paper. It is critical that the ice cream extrude uniformly and be cut cleanly for the process to work effectively.

A common problem that the ice cream sandwich manufacturer faces is tailing. This happens when the ice cream does not cut cleanly as the cookies advance. The result is a trail of ice cream being deposited on the wrong side of the cookies. In extreme cases the problem can cause the filling equipment to malfunction, causing down time. The reason for tailing can be as simple as incorrect temperatures from the freezer or as complex as a seasonal change in the fat used for the ice cream.

A good stable structure that is semirigid is necessary for the ice cream to cut cleanly and flow without mixing. Proper extrusion qualities are in large part a result of the state of the fat in the ice cream. Air bubbles in the ice cream are stabilized by agglomerated fat. The air cells are embedded in a semirigid structure formed by partially agglomerated fat (Goff 1997), ice crystals, and the freeze concentrated serum. In non-fat products, this structure is weakened by the absence of this fat structure. In reduced fat novelty applications one must rely upon ice crystals and the unfrozen serum to stabilize the structure. Since the fat in frozen products is concentrated by freezing along with the other components, it is only necessary for a small amount of fat to be present to have a large effect on the extrusion properties of the mix. Emulsifiers and hydrocolloid stabilizers can help build back the structure of frozen desserts when the fat is removed.

In products containing fat it is important to formulate and process the mix to maximize the structure in the extruded mix. Part of the structure in these products is formed from partially agglomerated fat. The emulsion state of the mix is a result of a complex interaction between fat globules and protein and other substances on the surface of the fat globules (Arbuckle, 1986). In the unfrozen mix, it is desirable for the fat to be stable enough to be pumped and agitated during storage and delivered to the freezer without churning. It is also desirable for the fat to resist creaming during storage. During the freezing process it is necessary for the fat particles to interact to form a semirigid structure (Arbuckle 1986). This structure is the result of the partial agglomeration of the fat particles forming three-dimensional structures surrounding the air in the mixture (Bollinger and others 2000). The ingredients used to control this are emulsifiers. In this application emulsifiers are not used to promote the formation of an emulsion but to partially destroy it. As with most phenomena in foods, the situation is not as simple as one would suppose at first. In this case the protein in the mix interacts with the fat globule, and it is this complex interaction that makes the products possible (Barfod and others 1991). It should be remembered that proteins, especially the caseins, have emulsifying properties (Walstra and Jenness 1984). Emulsifiers chosen for ice cream must have the ability to slowly displace the protein from the fat globule (Turnbow and others 1992). If this displacement occurs too fast, the mix will churn during processing. If it is too slow, excessive aging must take place. With too much protein on the surface of the fat globule, the globules will be prevented from agglomerating properly in the freezer and a weak structure will result. With too little protein on the surface of the fat globules the fat will churn and form large fat deposits in the freezer and pipelines from the freezer to the filler. Chunks of fat can be found in the ice cream in extreme cases of churning. Since extruded novelties are subject to extra stress during extrusion into shapes, it is especially important that proper fat agglomeration be achieved. Close control of the protein levels and type, and emulsifier selection will aid in achieving proper structure.

It is impossible, on a practical level, to observe the state of fat emulsions during processing. One characteristic that is observable however, is the surface appearance of the ice cream at the filler. The desired quality is called “dryness.” A dry, flat or eggshell like appearance on the extruding ice cream is desirable. The “dry” appearance is related to the amount of fat agglomeration (churning) that occurs in the ice cream freezer. Light is scattered by agglomerated fat. This light scattering causes the surface to take on the flat appearance. The amount and type of fat present, emulsifier system, freezer design and settings, mix aging, and formulation of the ice cream mix in general all contribute to the dryness quality. Often a small change in the fat source (seasonal changes in milk fat for instance) can cause a process that had been functioning well to fail completely. In these cases a change in emulsifier system is necessary to return to a good extrusion. Polysorbate 80 can often improve the extrusion qualities of a mix in this case. Where warranted, stabilizer suppliers can produce stabilizers that are specifically designed to produce good extrusion qualities. Table 75.4 contains formulas for ice cream-based extruded novelties.

The crystal structure of a dessert that is frozen, extruded, and cut into bars, more closely resembles a premium ice cream than similarly shaped novelties made by quiescently freezing a similar mix. Products made this way can command a premium price in the market. Extruded novelties need not be limited to simple rectangular bars. It is possible to coextrude several streams of frozen product using complex extrusion heads. Using this technology, many fanciful designs are possible. There are some limiting factors, however. Designers should avoid having a stream of frozen product where small

TABLE 75.4 Example Formulations for Ice Cream Based Novelties.

Component	Ice Milk for Ice Cream Bars (%)	12% Ice Cream for Bars (%)	Sherbet for Shells (%)	Ice Cream for Extruded Novelties (%)	Super Premium Ice Cream for Bars (%)
Milk fat	6	12	1.5	10	16
Milk solids nonfat	12	10.5	3	10	9.8
Sucrose solids	11	13	22	12	16
36DE corn syrup solids	7	4	7	5.45	–
Citric acid	–	–	0.5	–	–
Polysorbate 80	–	–	–	0.05	–
Stabilizer-emulsifier[a]	0.5	0.5	0.4	0.5	0.35
Total solids	36.5	40	34.4	38	42.15

[a]Use manufacturer's recommendations.

narrow areas and larger areas are within the same flow. It is difficult to maintain uniform flow in this situation. Equipment manufactures have been able to design equipment for this application with multiple flowstreams or cleverly designed extrusion heads that have largely overcome these problems.

One new technology that has made possible very detailed novelty products is cold extrusion (Hansen 2004). Cold extrusion technology involves following a conventional freezer with another heat exchanger, where the temperature is brought down to levels 7–10 degrees lower than the conventional freezer. This heat exchanger often takes the form of an extruder similar to that used to produce shaped cereal. The difference is that heat is removed from the product. This produces a very firm ice cream with a very fine structure and a low temperature (Danisco 2003). Using molds that can be split into several sections and injecting the ice cream into the mold enables incredibly detailed molded novelties to be made (Hansen 2004). Liquid nitrogen-cooled molds can help prevent product form sticking (Hansen 2004). The stiffness and lack of the need for extensive hardening using cold extrusion has made this possible. These solutions can be costly and the novelty developer will need to balance this cost with the desired complexity of the product.

75.5 THE CHANGEABLE NATURE OF NOVELTIES

Volatility is the nature of products like frozen novelties, as the demand for specific types of novelties will vary over time. Cartoon characters and the popularity of shapes and flavors come and go. A stroll down the aisle of any grocery store in the developed world will confirm this. It is one of the advantages of frozen novelties that the equipment is designed to be flexible. Molds can be changed, flavors and colors can be altered, and completely new products can be designed. It is important to keep closely allied with equipment and ingredient suppliers and to keep a close eye on the marketplace so that products can be developed as demand is generated and maintained.

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76

Yogurt

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76.1 Introduction 648
76.2 Definition of Yogurt 649
76.3 Yogurt Manufacture 649
76.3.1 Raw Ingredient Receiving and Quality Inspection 650
76.3.2 Milk Standardization 651
76.3.3 Culture Handling 652
76.3.4 Yogurt Mix Blending 653
76.3.5 Mix Homogenization and Pasteurization 654
76.3.6 Inoculation, Fermentation, and Cooling 655
76.3.7 Fruit, Flavor, and/or Color Addition 658
76.3.8 Packaging and Storage 658
76.3.9 Finished Product Quality Control 659
76.3.9.1 Safety and Regulatory Quality Control 659
76.3.9.2 Organoleptic Quality. Texture, Flavor, Color 659
76.3.9.3 Shelf Life Quality Control 660
76.4 Manufacture of Specialty Yogurt Products 661
76.4.1 Yogurt Drinks 661
76.4.2 Aerated Yogurt 661
76.4.3 Frozen Yogurt 662
76.4.4 Greek Yogurt 662
76.5 Chemical and Biological Changes During Yogurt Production and Shelf Life 662
76.5.1 Heat Treatment and Homogenization 662
76.5.2 Fermentation 663
76.5.3 Postfermentation Cooling and Storage 664

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

76.6 Yogurt Microbiology 664
76.7 Yogurt Nutrition 668
References 670

76.1 INTRODUCTION

This chapter will provide a brief overview of the science and technology of yogurt manufacture with particular emphasis on commercial practices in the United States. Information in this chapter will include a definition of the product category as well as methods for production and quality control, chemical, and biological changes during yogurt production, yogurt microbiology, and nutritional aspects of yogurt. An excellent source for more detailed information about yogurt is the comprehensive book by Tamime and Robinson (1999).

Yogurt is a traditional fermented milk food that has shown strong sales growth in the United States dairy grocery category during the past two decades. Production in the United States more than tripled between 1980 and 2002 (from 540 to 2100 million pounds) and per capita consumption has increased steadily (Fig. 76.1).

In the United States, the refrigerated yogurt category is segmented by nutritional value of product (full fat, low fat, nonfat, artificially sweetened, low calorie), by packaging format (single cup, large size, multipack cups, tubes, and bottles) and by the type of product (cup set, fruit on bottom, blended or stirred style, and beverage). The category may also be segmented by consumer benefit with offerings for snack yogurt, dessert or indulgent yogurt, drinking yogurt, and specific health benefit yogurt, among others. Yogurt for children introduced in the 1980s is a rapidly growing segment in the U.S. market. In particular, beverages based on yogurt, and yogurts with specific health benefits (such as low lactose or probiotic) are areas of tremendous current growth.

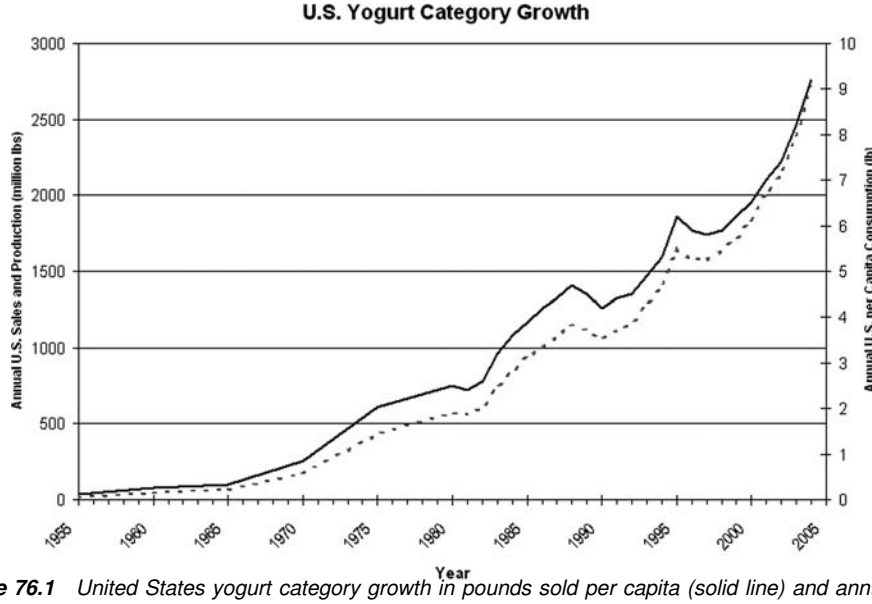


Figure 76.1 *United States yogurt category growth in pounds sold per capita (solid line) and annual dollar sales (dashed line) (International Dairy Foods Association 2005, Milk Industry Foundation 1980).*

76.2 DEFINITION OF YOGURT

The U.S. definition of yogurt can be found in the Code of Federal Regulations (CFR), Title 21, Volume 2, Parts 131.200, 203, and 206 (21 CFR 131.200–206 2003). In Europe, yogurt is defined by Codex Alimentarius Standard 243-2003 (Codex Alimentarius Standard 243-2003 2003). Generally, the definition of yogurt requires that it be a milk product that is fermented by the action of both *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Different countries have different regulations regarding acceptable additives, minimum required quantity of live organisms, and final pH or acidity. In the United States the CFR does not require specific numbers of viable bacteria to meet the identity of yogurt. However, the National Yogurt Association (NYA), a trade association of yogurt manufacturers, offers a "live and active" culture seal. Commonly found on refrigerated yogurt products, the presence of the "NYA live and active seal" guarantees 10^8 colony forming units per gram at the time of manufacture, and activity of the cultures at the end of shelf-life of the product. The milk fat content of yogurt is regulated by the CFR with definitions for yogurt, low fat yogurt, and nonfat yogurt (21 CFR 131.200–206. 2003).

76.3 YOGURT MANUFACTURE

The Grade A Pasteurized Milk Ordinance, enforced by the Food and Drug Administration (FDA), is the primary regulation guiding the manufacture of milk products in the United States (U.S. Department of Health and Human Services 2002). In Europe, each country defines its own standard yogurt manufacturing practices. Although there are traditional methods of yogurt manufacture, this chapter will cover only the commonly used practices for commercial yogurt production. The basic steps required for manufacture of yogurt include:

1. Raw ingredient receiving and quality inspection
2. Milk standardization
3. Culture handling
4. Yogurt mix blending
5. Mix homogenization and pasteurization
6. Inoculation, fermentation, and cooling
7. Fruit, flavor, and/or color addition
8. Packaging and storage

The general goal of yogurt production is to create a semisolid, long shelf-life food from perishable, fluid milk. Development and retention of the appropriate texture is critical to good yogurt quality. Yogurt mix preparation (particularly heat treatment and fortification), fermentation conditions (particularly culture type), and postfermentation handling all have a great impact on product texture. Each of these will be covered briefly in this chapter. For more detailed information, consult recent review articles (Jaros and Rohm 2003; Lucey 2004; Sodini and others 2004). A general scheme for the production of yogurt indicates that the process differentiation between cup set yogurt, stirred yogurt, and yogurt drinks generally occurs after pasteurization

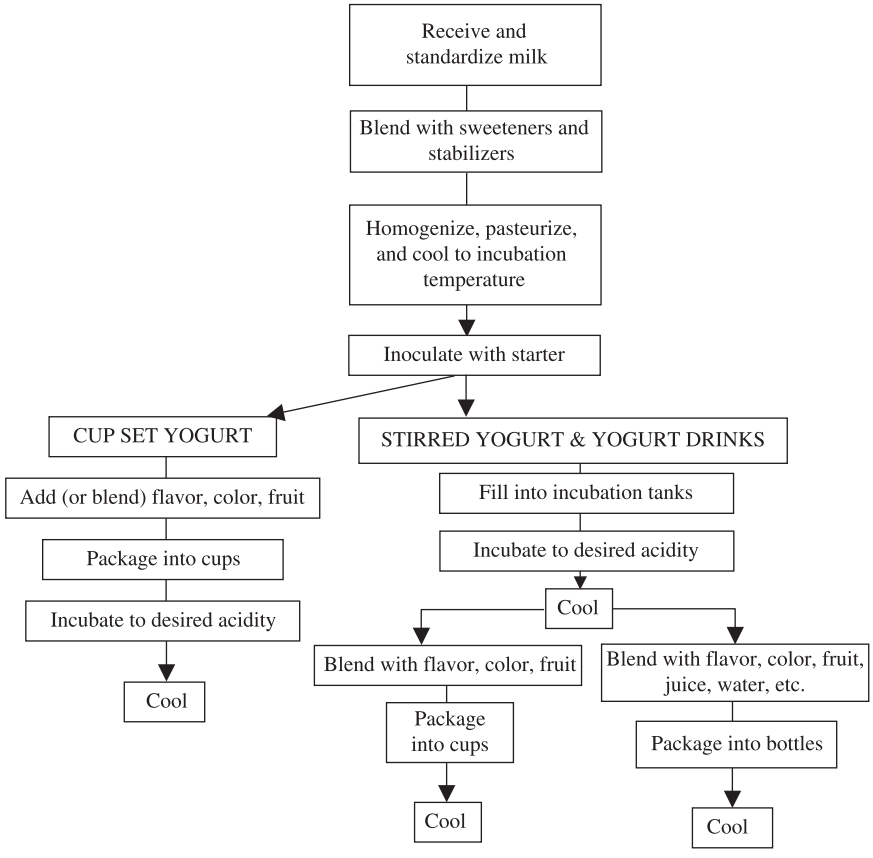


Figure 76.2 *A general scheme for yogurt manufacture. (Adapted from: Robinson and Tamime 1975, Lucey 2004, and Chandan 1982.)*

(Fig. 76.2). Typical equipment installations for stirred style and cup set yogurt can be found in Figures 76.3 and 76.4.

76.3.1 Raw Ingredient Receiving and Quality Inspection

According to U.S. regulation, incoming raw milk must be tested for antibiotics. This is usually done by a commercially available rapid method specific for a certain antibiotic or class of antibiotics or by a microbial inhibition method (Bishop and others 1993; U.S. Department of Health and Human Services 2001a). It is critical that milk be free of antibiotics as even low levels can inhibit the yogurt culture. The fat, protein, and total solids content can be determined by a standard method or calibrated rapid method (Bishop and others 1993). Compositional information is important when standardizing the yogurt mix. Raw milk is often clarified to remove dirt and unwanted materials such as somatic cells (Humphreys and Plunkett 1969; Puhan 1988). Clarification is commonly done in a centrifugal separator during milk fat content standardization, but is also sometimes accomplished with a dedicated clarifier. Clarification can induce rancidity in raw milk, so its use should be followed immediately by heat treatment (Humphreys and Plunkett 1969).

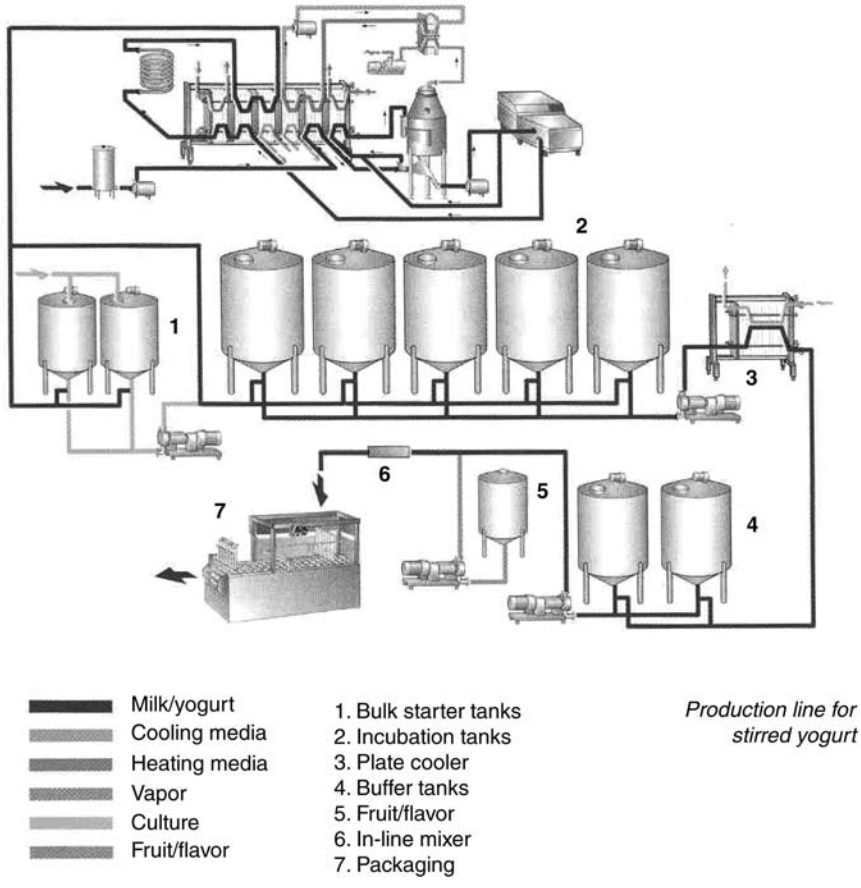


Figure 76.3 *Typical stirred style yogurt manufacturing installation. Reprinted with permission from Tetra Pak (Tetra Pak 1996).*

76.3.2 Milk Standardization

Milk is often separated into skim and cream components to allow standardization to the desired milk solids and fat content in the yogurt mix. A Pearson square calculation can be used to easily calculate the necessary mix of dairy ingredients to achieve a desired milk solids or fat level (Tamime and Robinson 1999). A dairy will generally utilize the most economical blend of ingredients to achieve the target composition. However, allowable milk sources may be limited by applicable regulations. Milk sources commonly used worldwide in the yogurt industry include raw milk, condensed milks, dry milk powders, milk protein concentrates, and caseinates. Because ultrafiltration (UF) removes lactose and some minerals, UF retentate is useful for making lower lactose, high protein products (Glover 1985; Puhan 1988).

Concerns important when utilizing raw milk include proper separation of raw and pasteurized stages, strict adherence to sanitary measures to prevent contamination from milk trucks entering the dairy plant, safe handling of raw milk to prevent microbial

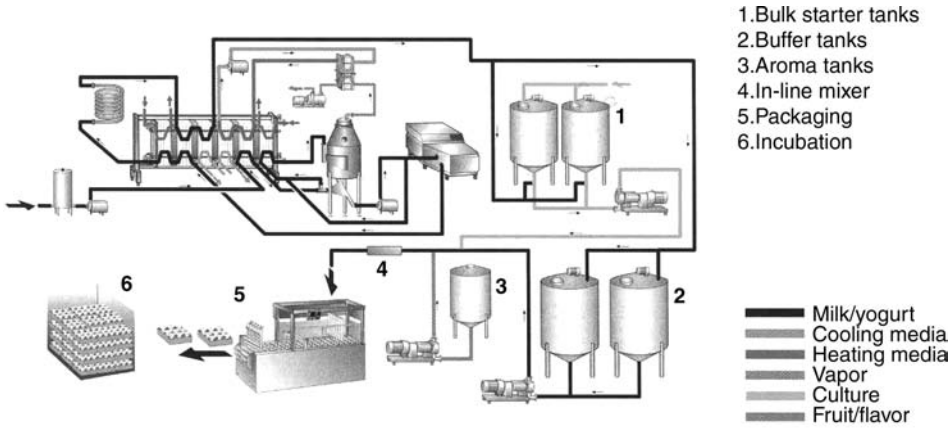


Figure 76.4 *Typical cup set yogurt manufacturing installation. Reprinted with permission from Tetra Pak (Tetra Pak 1996).*

growth, and gentle handling of raw milk prior to heat treatment to prevent lipase induced rancidity. If raw milk is agitated prior to heat treatment, the milk fat globule membranes can become disrupted and allow the active milk lipases to act on the fat, creating rancid off-flavors (Harper 1976; Bodyfelt and others 1988).

Yogurt mix is usually fortified with milk protein to increase gel strength and decrease susceptibility to syneresis (Davis 1975; Tamime and Deeth 1980; Harwalker and Kalab 1983; Kalab and others 1983; Modler and others 1983; Puhan 1988; Kesseler 1998; Schokoda and others 2001; Bhullar and others 2002; Remeuf and others 2003; Sodini and others 2004). Fortification can be accomplished by the addition of skim milk powder (often 1–3%), whey protein concentrate (often 1–2%), or ultrafiltration retentate. Alternatively, the mix may be concentrated by evaporation or ultrafiltration (10–15% concentration) (Puhan 1988; Lucey and Singh 1997). If total solids are higher than 22%, fermentation may be slowed due to increased osmotic pressure of the milk blend (Tramer 1973; Tamime and Robinson 1999). The method of fortification will impact finished product quality. For example, fortification with caseinates increases gel strength more than fortification with skim milk powder (Guinee and others 1995). Yogurt will generally contain 9–20 g total milk solids (including fat) per 100 g with the lower end typical of low fat yogurt (Tamime and Deeth 1980).

76.3.3 Culture Handling

Both *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are required to make yogurt. Few dairies maintain their own culture collection because of the expense associated with culture maintenance. Rather, yogurt cultures are generally purchased from culture manufacturers as deep-frozen concentrates or freeze dried powders, and stored at −40 to −57°C (−40–71°F) until use (Mocquot and Hurel 1970; Sellars 1989). Cultures may be the direct set type, or culture concentrates may be used at the dairy to create a bulk starter culture. Direct set (DS) cultures are a convenient way for manufacturers to maintain the target rods to cocci ratio, while also delivering desired counts of

adjunct microorganisms, such as *Lactobacillus acidophilus* or *Bifidobacterium* species. Freeze-dried or frozen DS cultures contain 10^7 to 10^9 colony-forming units per milliliter (Sellars 1989; Tamime and Robinson 1999). For DS cultures, the dairy might utilize a small tank for thawing and dilution of the culture prior to addition, or they may choose to add frozen culture to each batch in the fermentation tank. DS cultures generally exhibit slower fermentation than bulk starter cultures due to an extended lag phase (Tamime and Robinson 1999).

Dairies may create a mother culture in the laboratory that is used to inoculate milk to produce bulk culture. Although freeze dried cultures require subculturing to regain activity prior to bulk culture production, deep frozen cultures do not, and may be used for direct inoculation of bulk culture milk. Bulk cultures must contain sufficient viable organisms in the correct ratio, be free from yeast, molds, coliforms, bacteriophage, or other contaminants, and be capable of growth in the yogurt mix (Tamime and Robinson 1999).

Milk for bulk culture production is generally nonfat dry milk reconstituted to 9–12% milk solids not fat (MSNF) which has been heated to 85–95°C (185–203°F) for 30 min, then cooled to 37–43°C (99–110°F). This can occur in one vat that is jacketed with both steam and cold water lines. Thawed, concentrated culture is added to the milk, which is then incubated to the desired endpoint, commonly pH 4.4–4.6 or titratable acidity of 0.8%. The resulting bulk culture is agitated while being cooled to 40°F (Sandine 1979; Chandan 1982; Chandan and Shahani 1995). Milk that is used for bulk starter growth should be pasteurized at a high temperature with a long hold time in order to release growth factors, destroy pathogenic and competing organisms, reduce the oxygen content, release sulfhydryl compounds, lower the pH, denature whey proteins, and destroy inhibitory compounds (Humphreys and Plunkett 1969; Davis 1975; Sandine 1979).

It is most important that cultures be handled aseptically to prevent the introduction of unwanted contaminants that may grow in the milk. Although not a common problem, bacteriophage can attack yogurt microorganisms. Therefore, strain selection, strain rotation, and strict adherence to good sanitation practices are necessary to prevent culture failure due to bacteriophage attack (Chandan 1982; Sanders 1989).

76.3.4 Yogurt Mix Blending

Other ingredients are mixed with the standardized milk in a blending step. For sweetness, sugar is often added at this stage. Common sweeteners include crystalline or liquid sucrose, crystalline fructose and high fructose corn syrup. Sugar addition greater than 12%, and sometimes as low as 9%, will noticeably slow fermentation by changing the osmotic potential of the mix (Tramer 1973; Davis 1975; Puhan 1988; Chandan and Shahani 1995; Tamime and Robinson 1999). Stabilizers, though not required, are often added to reduce the susceptibility of the yogurt to spontaneous whey separation (syneresis) and to provide reset viscosity in stirred-style products. In addition, stabilizers add body and viscosity, create a firmer gel, and increase the ability of the gel to withstand refrigerated distribution (Humphreys and Plunkett 1969; Chandan 1982; Puhan 1988; Chandan and Shahani 1995; Tamime and Robinson 1999). Common stabilizers, and their typical use rates in the yogurt mix, include gelatin (0.2–0.4%), starch (1.2–2.0%), pectin (0.05–0.2%), agar (0.8–1.12%), carageenan (0.05–0.2%) (Puhan 1988), and (less commonly) locust bean gum, xanthan gum, and guar gum (Lucey 2004).

Hydrocolloids in yogurt can affect elasticity, viscosity, and stability of the coagulum. Some stabilizers will react with charged milk proteins. This can create phase separation,

graininess, and interfere with gel formation. These problems are sometimes evidenced by a less shiny, slightly grainy surface appearance (Dickinson 1998; Olson 2002). These stabilizers (pectin, guar, locust bean gum, sodium carboxymethylcellulose, gum arabic, and carageenan) should, therefore, be added only after fermentation and in small amounts (Puhan 1988; Dickinson 1998; Poppe 1999; Tamime and Robinson 1999) or in combination with other ingredients that minimize their reactivity. Caseinate addition will increase yogurt viscosity and reduce syneresis, but can result in a grainy texture at higher levels (greater than 0.3%). Modified starches (resistant to acid and shear) can provide yogurt viscosity. Stabilizers often require special handling, such as premixing with other dry ingredients, or hydration prior to sugar addition to ensure full functionality. High-methoxy pectin is recommended for acidified dairy beverages due to its ability to stabilize milk proteins at low pH, while low-methoxy pectin is recommended to add viscosity to yogurt (Puhan 1988; Dickinson 1998; Leskauskaite and others 1998; Olson 2002). Pectin can interact with exopolysaccharides produced by yogurt cultures to increase viscosity and reduce syneresis (Olson 2002). Some stabilizers (such as some types of pectin or alginate) are calcium reactive and, therefore, should be added only in small amounts or with chelators. Gelatin is effective both at reducing syneresis and adding body to finished yogurts (Modler and Kalab 1983; Modler and others 1983; Modler and Kaleb 1983; Guinee and others 1995). Typically, a 100–150-bloom gelatin is used (Imeson, Thickening and Gelling Agents for Food 1997).

Reduced calorie yogurts are generally nonfat and use high intensity sweeteners such as aspartame or sucralose to replace sugar. Aspartame is not heat stable, so it is generally added post-fermentation, often via the fruit preparation.

Preservatives, such as potassium sorbate or sodium benzoate are sometimes added to yogurt to inhibit yeast and mold growth, but may not be allowed by local regulation (Tamime and Robinson 1999).

Ingredient addition is most often accomplished by use of a high-speed mixer, such as Multiverter or Liquiverter made by APV (APV, Denmark). In smaller operations, dry ingredients may be added gradually through an inline mixer, such as a Tri-blender (Alfa Laval, Sweden). Proper mixing ensures sufficient hydration of dry milk solids and stabilizers while minimizing incorporation of air.

76.3.5 Mix Homogenization and Pasteurization

Homogenization reduces fat globule size to prevent creaming of fat in the finished product, improves yogurt texture and stability, denatures some of the whey protein, affects stabilizer incorporation and functionality, and can increase the viscosity of higher fat yogurts (Humphreys and Plunkett 1969; Puhan 1988; Tamime and Robinson 1999). Some yogurts are made with unhomogenized milk to intentionally provide a cream layer on top of the yogurt. Homogenization can improve the body of lower solids yogurt, but has less effect on higher solids yogurt, so homogenization pressures should be tailored to the specific yogurt mix (Puhan 1988). Typically, yogurt mix is heated to 55–80°C (131–176°F), then homogenized in one or two stages at 7–20 mPa (1000–2900 psi) (Puhan 1988; Lucey and Singh 1997; Tamime and Robinson 1999). Homogenization after pasteurization can improve yogurt consistency, but is generally carried out before pasteurization in order to minimize the risk of contamination (Puhan 1988). Pasteurization must occur either prior to or immediately after homogenization to destroy lipases that would

otherwise create rancid off-flavors in the raw homogenized mix (Puhan 1988). Homogenization also increases product susceptibility to light activated off-flavor (Puhan 1988).

Pasteurization is carried out to destroy any pathogenic bacteria in the mix. An extended heat treatment is applied to yogurt milk to denature the whey proteins. The heat treatment also serves to reduce the number of competitive microorganisms, inactivate natural milk enzymes such as lipases, hydrate stabilizers, reduce the oxidation-reduction potential (favoring microaerophilic growth), and produce growth factors for lactic acid bacteria (Davis 1975; Puhan 1988; Chandan and Shahani 1995; Tamime and Robinson 1999). The extended heat treatment ensures 80–95% whey protein denaturation in the yogurt mix (Puhan 1988; Lucey and others 1997), which enhances yogurt texture and reduces susceptibility to syneresis (Grigorov 1966b; Dannenberg and Kessler 1988) as discussed later in this chapter.

Yogurt mix is typically heated to 80–85°C (176–185°F) for 30 min or 90–95°C (194–203°F) for 5 min (Humphreys and Plunkett 1969; Davis 1975; Lucey and Singh 1997). Homogenized, pasteurized milk blends can be held chilled until ready to use, providing an opportunity to check mix total solids and composition (Humphreys and Plunkett 1969). Insufficient heating will lead to a weak gel structure after fermentation, while excessive heating can lead to decreased viscosity and graininess (Sodini and others 2004).

Pasteurization as well as the extended heat treatment are most often carried out in a plate heat exchanger equipped with a long holding tube. However, in multiproduct plants the extended heat treatment is often accomplished following HTST pasteurization directly in the fermentation tank. After pasteurization, the mix is cooled to the fermentation temperature, typically 43–45°C (109–113°F) (Lucey and Singh 1997).

76.3.6 Inoculation, Fermentation, and Cooling

Fermentation can take place directly in the yogurt cup (set-style or cup-set) or in a fermentation tank resulting in a stirred style yogurt. Bulk starter is added to the warm yogurt mix at 2–3% (Davis 1975; Lucey and Singh 1997), while DS starter culture is added at a much lower rate, typically 0.02%. Starter culture can be injected inline as the base mix is pumped or added batchwise to a tank. After thorough mixing to incorporate starter, it is important that the mix be incubated quiescently during fermentation as agitation could result in graininess, lumpiness, or catastrophic precipitation of the proteins.

The fermentation temperature will affect product quality. A lower fermentation temperature favors the growth of *Streptococcus thermophilus* while a higher temperature favors the growth of *Lactobacillus bulgaricus* resulting in a different texture and flavor in the finished product (Mocquot and Hurel 1970; Tamime and Robinson 1999). Favoring the growth of *Lactobacillus bulgaricus* will usually result in a more acidic yogurt. Some yogurt cultures are characterized as "ropy" due to their production of exopolysacchrides. A lower break pH (final fermentation pH) will lead to a higher rods : cocci ratio (Kneifel and others 1993). Maintaining the fermentation temperature between 40–45°C (105–115°F) promotes growth of both cultures. The two cultures grow associatively, each benefiting from the growth of the other, which is discussed in more detail later in this chapter. The generally recommended temperature for optimal growth of the two cultures together is 42.5°C (108.5°F) (Mocquot and Hurel 1970), with slower growth at both higher and lower fermentation temperatures (see Fig. 76.5).

When the yogurt reaches the desired endpoint, as measured by titratable acidity or pH, the yogurt is cooled. The fermentation endpoint (fermentation break) will be reached in

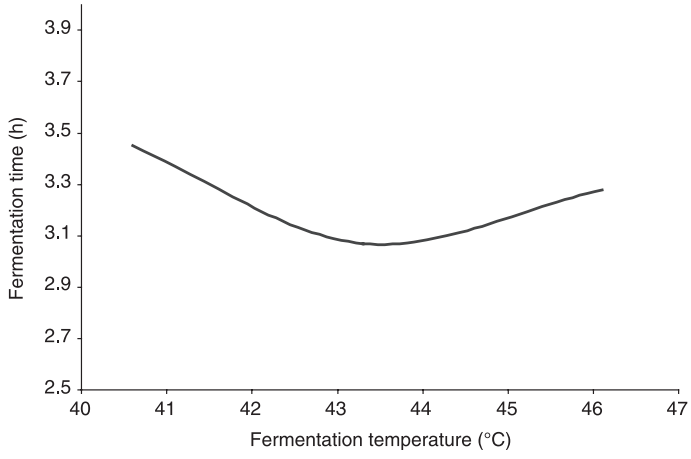


Figure 76.5 *The effect of temperature on fermentation time of a proprietary mixed culture of S. thermophilus and L. bulgaricus in milk. The optimum temperature for this culture blend is approximately 43 °C. Printed with permission from General Mills, Inc. (Maeve Murphy and Kyle Long, unpublished data).*

2–5 h, depending on the culture used and the mix composition. Because acidity will continue to develop as the yogurt is cooled, the endpoint must be adjusted to compensate for this continued fermentation (Tramer 1973). Depending on the rate of cooling, this process should begin when the yogurt reaches pH 4.4–4.8 in order to reach a final pH of 4.3–4.5 at the end of cooling. For cup set yogurt, sampling is important, as cups on the bottom of a pallet may have cooled more during pallet stacking, leading to a different fermentation rate compared to cups on the top of the pallet.

To cool cup set yogurt, pallets of cased product are either passed through cooling tunnels or placed in front of large cooling fans. Cases should have holes in them to facilitate the flow of cold air through the packaging and accelerate cooling. Bulk set yogurt can be cooled in the tank, if the tank is jacketed with chilled water and equipped with an agitator. However, cooling through a plate heat exchanger is more common. In the United States, yogurt must be cooled to less than 45°F (7°C) prior to shipping to meet standards of the Pasteurized Milk Ordinance (U.S. Department of Health and Human Services 2001).

Typically, bulk set yogurt is cooled to 10–21°C (50–70°F) to slow culture activity and, therefore, acid production. Fruit and flavor is added, the product is packaged and then cooled to 2–5°C (36–41°F). This two-step process helps increase yogurt viscosity by minimizing damage to the protein coagulum (Tamime and Robinson 1999; Olson 2002) (see Fig. 76.6).

In some instances, yogurt is cooled to 2–5°C (36–41°F) prior to the addition of fruit and flavor, and is then packaged and warehoused without need for further cooling. The coagulum is more likely to suffer textural damage when pumped at these lower temperatures (Vedamuthu 1991b; Tamime and Robinson 1999; Sodini and others 2004). The gel can recover some viscosity, but recovery is not full (Guinee and others 1995; Sodini and others 2004). For maximum viscosity and the most stable product, Maiocchi (2003) recommends a three step cooling process whereby yogurt is cooled to 25–30°C (77–86°F) prior to cup filling, then quickly cooled in the package to 10–15°C (50–59°F) in a blast cooler followed by slow cooling to 4°C (40°F) over 24–48 h.

In the case of stirred style yogurt, the coagulum is pumped or gravity fed from fermentation tanks to packaging fillers, and is mixed with fruit, flavor and color during the

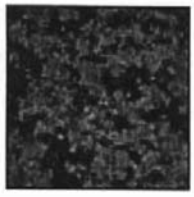
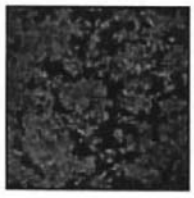
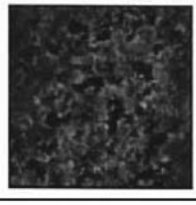
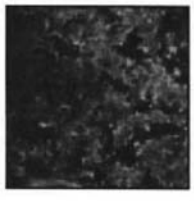
Filling temperature	Description of yogurt
10°C 	Relatively low mouthfeel Watery consistency Tendency to whey separation Looser protein structure
15°C 	Still relatively low mouthfeel Still watery consistency Reduced tendency to whey separation Looser protein structure
20°C 	Higher mouthfeel Higher viscosity Very little tendency to whey separation
25°C 	Even higher mouthfeel Even higher viscosity Very little tendency to whey separation

Figure 76.6 *Effect of filling temperature on yogurt structure (Olson 2002). Printed with permissioin from Danisco USA Inc.*

process. Stabilizers can make the coagulum more robust by contributing to the gel recovery. To minimize the mechanical stress on the yogurt gel, positive displacement pumps such as lobe-type rotary pumps, wing type rotary pumps, or screw type pumps (Tamime and Robinson 1999) are utilized. Choosing the right pump, running a lower pump speed, minimizing the distance of pumping, utilizing special plate heat exchangers with larger gaps between plates, as well as avoidance of pipe diameter reductions and 90° elbows in piping all help to minimize mechanical damage (Tamime and Deeth 1980; Vedamuthu 1991b; Tamime and Robinson 1999; Maiocchi 2003). Pumps optimally should be run at 60 rpm, and no more than 100 rpm to preserve yogurt texture (Maiocchi 2003). When a smoother texture is desired in the yogurt, shear can be applied by using a smoothing screen or shear valve (Tamime and Deeth 1980; Lucey 2004). Typical shear valves are throttling valves which may be adjusted to constrict the flow of yogurt through the line, creating back pressure. Screens may be as a simple as a filter/strainer made of perforated metal inserted into the piping. Both shear valves and screens smooth the yogurt by increasing mechanical shear on the coagulum.

76.3.7 Fruit, Flavor, and/or Color Addition

For cup set yogurt, fruit, flavor, and color are added prior to fermentation. Fruit is filled directly into cups for fruit on the bottom yogurt. For blended or stirred style yogurt, these ingredients are added to the yogurt after cooling either continuously via inline injection or batchwise by direct addition to the tank. Heat treatment of fruit for addition will help ensure product safety and minimize the possibility of contaminating the yogurt with yeast and mold. Pectins in fruit preparations can sometimes lead to product defects due to interactions with the calcium in the yogurt. The interface between the fruit and the yogurt in fruit on the bottom products, is particularly problematic. Water activity differences between the fruit and yogurt layers can lead to water migration issues.

Fruit preparations for yogurt are typically comprised of fruit (15–60%), sweetener (0–40%), stabilizers (3–5%), flavor, color, preservatives (potassium sorbate and/or sodium benzoate), acid, and water (Chandan 1982; Hegenbart 1990). The preparation is heat treated, and aseptically filled into stainless steel totes or smaller packaging. Final fruit prep is usually 60–65% solids and 40–50 degrees Brix (Hegenbart 1990; Chandan and Shahani 1995). Viscosity is critical for fruit on bottom style yogurt to provide clear delineation between fruit and yogurt, and to prevent fruit from floating to the top of the unfermented base. Stability against microbial spoilage is provided by the high solids, the low pH (less than pH 4) and any preservatives in the preparation. Heat treatment should be severe enough to decrease microbial load and activate gums or cook starch, but as gentle as possible to preserve fruit quality. Fruit preparations typically have a 1-year shelf life when stored refrigerated, although 6 months is a common limit to ensure higher quality. Fruits used in preparations are usually frozen with sugar, because straight-pack (frozen without sugar) fruits tend to be less firm in the preparation. Viscosity is often measured using a Bostwick consistometer with a target of 5 units (cm) in 30 s at 24°C (75°F) being typical (Chandan and Shahani 1995). Fruit preparations must have a pH that is compatible with yogurt (generally pH 4), color that will show no or minimal migration to the yogurt, desired viscosity, and meet strict microbiological standards (generally less than 10 CFU per gram for yeast, mold, and coliforms) (Chandan 1982; Hegenbart 1990; Klink 2003).

Flavor and color ingredients also need to conform to strict microbiological specifications. Typically, the requirement is <10 CFU/mL yeast, mold, and coliforms. Ingredients added after pasteurization need to be handled in a sanitary manner to prevent possible contamination of the yogurt.

Accurate addition of fruit, color, and flavor is important for consistent yogurt quality. Mass flow meters are often used to provide a controllable process. To minimize damage to fruit, positive displacement pumps are generally used to pump fruit preparation. Pumps that accurately dispense small amounts, such as peristaltic (or hose type) pumps, are required for flavor and color addition (Tamime and Robinson 1999).

76.3.8 Packaging and Storage

Plastic is the packaging material of choice for yogurt with polypropylene and polystyrene cups being the most common. Yogurt packaging material must be durable, allow a hermetic seal, protect the product from moisture loss, and be easy for the consumer to use.

Oxygen transmission rates are also an important consideration, both for survival of sensitive cultures and for stability of added vitamins. Both thermoformed and injection molded plastic containers are used. Antistatic coatings should be included in packaging material to minimize the likelihood of dust or airborne microbes being attracted to the cups (Humphreys and Plunkett 1969). Filling into packaging should take place with a minimum of air incorporation to maintain best product quality. In the United States finished products must be stored at less than 45°F to meet the requirements of the Grade A Pasteurized Milk Ordinance (U.S. Department of Health and Human Services 2001).

76.3.9 Finished Product Quality Control

Quality control must ensure the finished product is safe, meets all regulatory requirements, provides the desired organoleptic qualities, and maintains those qualities through the product shelf life.

76.3.9.1 Safety and Regulatory Quality Control. Closely monitoring the pasteurization step and all postpasteurization ingredient addition points, as well as regular environmental monitoring for pathogens and undesirable organisms are key to ensuring product safety and quality. To meet applicable regulatory requirements, the milk blend or product should be tested for total solids and fat content. The finished product should be tested for yeast, mold, and coliform contamination, as well as for lactic acid bacteria. Methods for these are outlined in *Standard Methods for the Examination of Dairy Products* (Marshall 1993).

76.3.9.2 Organoleptic Quality. Texture, Flavor, Color. Monitoring product quality is important for maintaining consumer satisfaction. Evaluation of texture, flavor, and color are typically done at the time of production by the processor.

Product texture is closely monitored. Measurement of gel firmness is usually with a penetrometer, a heliopath rotational viscometer with a t-bar spindle (Brookfield, USA), or a Haake type viscometer (Thermo Electron Corp., USA). Apparent viscosity of blended yogurts is measured with a rotational viscometer usually at a single, predetermined shear rate (Tamime and Robinson 1999; Jaros and Rohm 2003).

Failure to ferment is characterized by a lack of acid production and failure to form a protein gel. This can be caused by the presence of inhibitory substances such as antibiotics or sanitizers, by the presence of bacteriophage, by the use of mastitic milk, or by the presence of short chain free fatty acids (Humphreys and Plunkett 1969; Tramer 1973; Davis 1975; Sanders 1989; Tamime and Robinson 1999). *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are very sensitive to antibiotics, particularly the former (Kosikowski and Mocquot 1958). Mixed strains used in yogurt manufacture may show slowed fermentation, rather than stopped fermentation after bacteriophage attack (Sanders 1989). While bacteriophage is traditionally less of a problem in yogurt plants than cheese plants (probably because yogurt plants are not separating and storing large amounts of whey (Davis 1975), bacteriophage will attack both *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Therefore, proper selection of phage resistant strains, proper sanitation and a culture rotation program will help prevent phage attack (Chandan 1982; Sanders 1989; Tamime and Robinson 1999). Sanitation to prevent phage attack must include sanitizers effective against phage, such as chlorine-based compounds.

Lumpiness and graininess are two common textural defects in yogurt. Lumpiness is characterized by large protein aggregates in yogurt, often visible as white lumps, and graininess is smaller particles. Both defects can be caused by excessive acid produced too quickly, the use of rennet, excessive amounts of starter culture, agitation, or vibration during fermentation, incorrect use of stabilizers or having an excessive whey protein to casein ratio (Humphreys and Plunkett 1969; Davis 1975; Lucey and Singh 1997; Remeuf and others 2003; Lucey 2004; Sodini and others 2004). Lumpiness can be corrected by pumping the yogurt through a screen or mesh filter; graininess is an irreversible defect.

Ropiness is a defect characterized by a stringy, mucilaginous texture. This is most often a result of excessive production of exopolysaccharides by the yogurt culture, a culture characteristic that may be desirable in moderation (Davis 1975). This defect can be avoided by proper strain selection and maintenance of the optimum fermentation temperature (Davis 1975).

Weak gel in the finished product cannot be corrected, so it must be prevented. Common causes of a weak yogurt gel are low milk solids, improper selection or batching of hydrocolloids, low pH of yogurt mix during processing, overly high homogenization pressure, insufficient hold time during heat treatment, improper fermentation temperature, high break pH, improper starter culture ratio or excessive shear after fermentation. Monitoring total solids prior to yogurt mix processing can help prevent batching or dilution errors. Improper sampling during fermentation can lead to inadvertantly breaking the yogurt at a high pH. Sampling to monitor fermentation should be collected in the center of the fermentation vessel and not in a previously sampled location.

Although acid enhances fruit flavors, it can detract from nonfruit flavors which are currently gaining popularity in yogurt. Acidity is measured by either titratable acidity or pH measurement (Marshall 1993). Typical yogurt flavor is characterized by acetaldehyde (23–55 ppm), with little or no diacetyl produced by yogurt cultures (Humphreys and Plunkett 1969; Tramer 1973; Rašić and Kurmann 1978; Chandan 1982). Over acidification results in a flavor defect characterized by a sour taste, syneresis, lower viable bacterial counts, and the accumulation of D(−) lactic acid. It may be caused by high storage temperature, incorrect starter strain, delayed cooling after fermentation or excessive starter culture (Humphreys and Plunkett 1969; Kneifel and others 1993).

Finished product flavor should be checked regularly. Some yogurt flavor defects are bitter (can be caused by incorrect strains, sporeformer growth, excessive stabilizers), unclean (can be related to poor sanitation or off-flavors in milk supply), and flat (can be caused by low incubation temperature, under fermentation, or inhibition of fermentation) (Humphreys and Plunkett 1969).

Yogurt, like milk, is susceptible to light activated off-flavor (sunlight flavor), and product should, therefore, be protected from light (Humphreys and Plunkett 1969; Bosset and others 1995; Tamime and Robinson 1999).

Color may be measured using a colorimeter (such as a Hunter Colorimeter, HunterLabs, USA) or by comparison to color standards such as Pantone or Munsell.

76.3.9.3 Shelf Life Quality Control. Yeast and mold control are critical to product quality because the growth of one or both of these is the usual cause of product failure at the end of shelf life. These microorganisms can originate from airborne or surface contamination, the latter being the more common source (Tamime and Robinson 1999). Regular testing of equipment and building surfaces will help identify potential problem areas early. Positive air pressure and high efficiency particulate air (HEPA) filtration in

the filling room will minimize airborne mold and yeast contamination. Contaminated starter culture, poorly cleaned filters, and ingredients added postpasteurization can also contribute yeast and mold (Vedamuthu 1991c). Yeast problems can be minimized by thoroughly heating milk and fruit preparations, avoiding reopening of fruit containers, thorough cleaning of equipment, sanitary ingredient handling and thorough sanitization of equipment prior to use (Davis 1975).

Gassy defects are most likely caused by yeast contamination and growth (Humphreys and Plunkett 1969). Yogurt cultures are homofermentative and the introduction of heterofermentative lactic acid bacteria can also lead to defects such as non-characteristic flavor and gassiness (Rašić and Kurmann 1978).

Whey separation is a defect caused by syneresis, a contraction of the coagulum that leads to exudation of whey. This may by minimized by applying sufficient homogenization, increasing the milk solids, adding a stabilizer (if allowed by applicable regulations), avoiding excessive or insufficient acid production, heating sufficiently to denature whey proteins, avoiding excessive starter addition, and avoiding containers with sloping walls (Humphreys and Plunkett 1969; Harwalker and Kalab 1983; Lucey and Singh 1997; van Vliet and others 1997; Tamime and Robinson 1999; Pereira and others 2003). Susceptibility to whey separation is often measured by gravitational methods (Jaros and Rohm 2003).

76.4 MANUFACTURE OF SPECIALTY YOGURT PRODUCTS

76.4.1 Yogurt Drinks

Yogurt drinks can either be yogurt that has a low viscosity, or a blended product that is a mixture of yogurt, and/or water, milk, and fruit juice. For drinkable yogurt, an intentionally thin yogurt is created by lowering milk solids, decreasing stabilizer content and/or applying extra shear after fermentation. To manufacture yogurt drinks, other ingredients (such as fruit juice) are mixed with yogurt typically followed by a blending step that may introduce significant product shear (such as homogenization) (Tamime and Deeth 1980; Berry 2002; Leporanta 2002).

Yogurt drinks often show whey separation and require shaking prior to consumption. Higher viscosity beverages show less separation than lower viscosity beverages. The use of high methoxy pectin to stabilize the proteins can help minimize the separation and create beverages that do not separate and have a smoother texture (Leskauskaité and others 1998; Danisco Cultor 2001; Olson 2002). Generally, other stabilizers such as modified starch or gelatin are used in combination with pectin.

Kefir is processed similarly to yogurt drinks, but fermented with a combination of yeast and bacteria. Cultures identified from traditional kefir include *Saccharomyces kefir*, *Torula kefir*, *Lactobacillus caucasicus*, *Leuconostoc* spp., and lactic acid streptococci, with yeasts representing 5–10% of the culture present (Kosikowski 1977). The presence of yeasts traditionally leads to a slight carbonation and different flavors. Products currently found in the U.S. marketplace rarely show carbonation.

76.4.2 Aerated Yogurt

This product is created by aerating the yogurt after fermentation and cooling (Klink 2003). Aerating equipment such as Oakes (E.T. Oakes, Inc., USA), Tanis (Tanis Food Tech, M.J. Meijer Aeration B.V., Netherlands) or Mondo (Haas-Mondomix BV, Netherlands) may be

used with the injection of an inert gas such as nitrogen or carbon dioxide to minimize oxidation potentially caused by the incorporation of air. The amount of gas added, calculated as overrun, ranges from 20% to 100% and can be adjusted to reach desired product attributes. To create a stable foam, stabilizer systems are required and can contain an emulsifier.

76.4.3 Frozen Yogurt

Frozen yogurt is a product that is a hybrid between ice cream and yogurt. Typically a frozen yogurt will contain 1.5–2% milk fat, 13–15% MSNF, 7–10% sucrose, 4–5% corn syrup solids (24–26 DE) and 0.15–2% stabilizers (Chandan 1982). A portion (generally 10–20%) of the frozen yogurt mix is fermented to pH 3.9 or a titratable acidity of 0.3%. This mix is blended with fruit, sugar, flavor, color, and frozen like ice cream (Chandan 1982; Marshall and others 2003). More information about frozen yogurt will be found in the ice cream chapter of this text.

76.4.4 Greek Yogurt

Greek style yogurt is traditionally thick, like a sour cream, and is produced by removing a portion of the whey from yogurt to increase the solids to 22–26% (Stringer and Dennis 2000). Usually a paterurized cream is added back to the strained yogurt to adjust the final fat content. Commonly, a small amount of sheep's milk may be added to provide characteristic aroma.

76.5 CHEMICAL AND BIOLOGICAL CHANGES DURING YOGURT PRODUCTION AND SHELF LIFE

76.5.1 Heat Treatment and Homogenization

Milk for yogurt production is subjected to a longer heat treatment than required to pasteurize the milk in order to denature the whey proteins. Denatured whey proteins, particularly beta-lactoglobulin, interact with the kappa casein or other whey proteins (Zittle and others 1962; Kalab and others 1983; Mottar and others 1989; Vasbinder and de Kruif 2003). This influences the pH of gelation, the size of the micelles and the strength of the resulting yogurt gel (Lucey and Singh 1997; Horne 1999; Anema and others 2004; Femelart and others 2004). Yogurt created with added whey protein has fewer fused micelles, more intermicellar spaces, and less syneresis than that made without added whey protein (Modler and Kalab 1983). The association of whey proteins with kappa casein is very pH dependent with more association at pH 6.5 vs. pH 6.7 (Anema and others 2004). This surface adherence shows up in electron micrographs as a fuzzy or spiky micelle surface (Kalab and others 1982; Lucey and others 1998) and leads to a more complex network with more interstitial spaces, and, therefore, increased water holding capacity (Pereira and others 2003). In unheated milk, micelles form coarse clusters during fermentation with larger interstitial spaces creating a gel more susceptible to syneresis (see Fig. 76.7) (Kalab and others 1983).

Because casein micelles exhibit a pH of maximum heat stability (pH 6.6), manufacturers of yogurt need to maintain the pH of the yogurt mix in an area of heat stability (pH 6.5–6.7) (Singh and Creamer 1992). If the mix pH is higher or lower than optimal, the caseins can

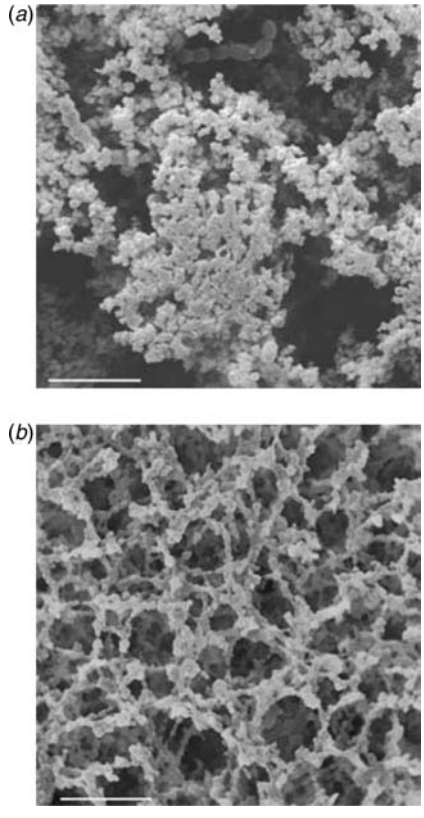


Figure 76.7 *Structure of yogurt gels from heated and unheated milk. Reprinted with permission from M. Kalab. Unheated milk, A. Heated milk, B. Bar shown is 5 micrometers in length. (From series taken at the time of Kalab and others 1983.)*

preprecipitate. This will be evidenced as high viscosity out of the heat exchanger, and will produce an inferior coagulum during fermentation (if coagulation is achieved at all).

Casein micelles are also sensitive to the calcium phosphate balance of the milk. Added ingredients that affect this balance, including water with a high mineral content, added calcium containing ingredients, or calcium chelating ingredients such as sodium citrate, will affect the stability of the micelle to heating (Singh and Creamer 1992).

The homogenization step during heat treatment also effects the final properties of the gel by increasing firmness. It appears that the effect is predominantly due to the reduction in size of the fat particle. While this results in an increase in the fat surface area and an increase in the amount of protein at the fat interface, the mechanism for the increased firmness is thought to be due to the formation of a finer gel network (i.e., one that is not disrupted by large fat globules) (Kesseler 1997).

76.5.2 Fermentation

During fermentation, the lactic cultures digest lactose and produce lactic acid. This gradual acid production lowers the pH. As the pH is decreased from about 6.7 to 5.3, the calcium phosphate balance changes as more colloidal (micellar) calcium phosphate

becomes soluble (Guinee and others 1993). The charge on the casein micelle starts to change becoming less negatively charged. As the milk reaches pH 4.6, the isoelectric point of casein, the micelles have a net neutral charge, losing the electrostatic interactions that helped prevent micelles from aggregating (Tamime and Robinson 1999). In heated milk, micelles start forming clusters and short chains at about pH 5.3. Whereas rennet gels are formed from clumps of casein micelles, acidified milk gels such as yogurt are formed from short chains or clusters of casein micelles and whey proteins. This is partly due to the interference of the denatured whey proteins on the surface and partly due to the differences in the coagulation mechanism.

The presence of denatured whey proteins shifts the gelation pH upward (Grigorov 1966a; Kalab and others 1983; Lucey and others 1997, 1999; Vasbinder and others 2001; Graveland-Bikker and Anema 2003). In model systems of casein and heated whey protein, gelation started at pH 5.8–6.2 with rapid gel strength increase at pH 5.3 (O'Kennedy and Kelly 2000). This leads to gel formation when only a portion of the colloidal calcium phosphate is solubilized.

76.5.3 Postfermentation Cooling and Storage

During refrigerated storage the cultures, particularly the lactobacilli, continue to produce acid (Tramer 1973). The majority of the acid is produced in the first seven days (Beal and others 1999). As yogurt ages, cultured dairy flavors increase, fruit flavor decreases, and sourness increases. Mold and yeast may grow if present. Whey separation may occur. All of these changes will accelerate as the temperature increases, so cold storage is important for maximizing the shelf life of yogurt. In the United States yogurt has a shelf life of 14–90 days from manufacture to the "sell by" date. This broad range is partly due to the fact many yogurts in the United States contain potassium sorbate to prevent yeast and mold growth. In the absence of yeast and mold growth, yogurt shelf-life is limited by flavor or appearance changes. Pasteurized or heat treated yogurt, which is heated after fermentation, will have a longer shelf life, but no live and active cultures.

76.6 YOGURT MICROBIOLOGY

Streptococcus salvarius subsp. *thermophilus* (also called *Streptococcus thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (also called *Lactobacillus bulgaricus*), are both thermophilic, homofermentative lactic acid bacteria (Hardie 1986; Kandler and Weiss 1986). *Lactobacillus acidophilus* is also included in many commercial yogurts sold in the United States. Other microorganisms are sometimes added to yogurt for nutritive effects, and will be discussed separately in the nutrition section of this chapter.

Because lactobacilli have complex growth requirements, growth media used for plating these organisms must be carefully chosen. Lactobacilli require low oxygen, fermentable carbohydrate, protein, protein breakdown products, nucleic acid derivatives, fatty acids, minerals, and some B-vitamins (Mital and Garg 1992). MRS broth and M-17 agar are the usual enumeration media for lactobacilli and streptococci, respectively (de Man and others 1960; Terzaghi and Sandine 1975; IDF 1983; Sellars 1989; Vedamuthu 1991a; Frank and others 2003). However, MRS agar is not useful for differentiating between *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* (Speck 1978).

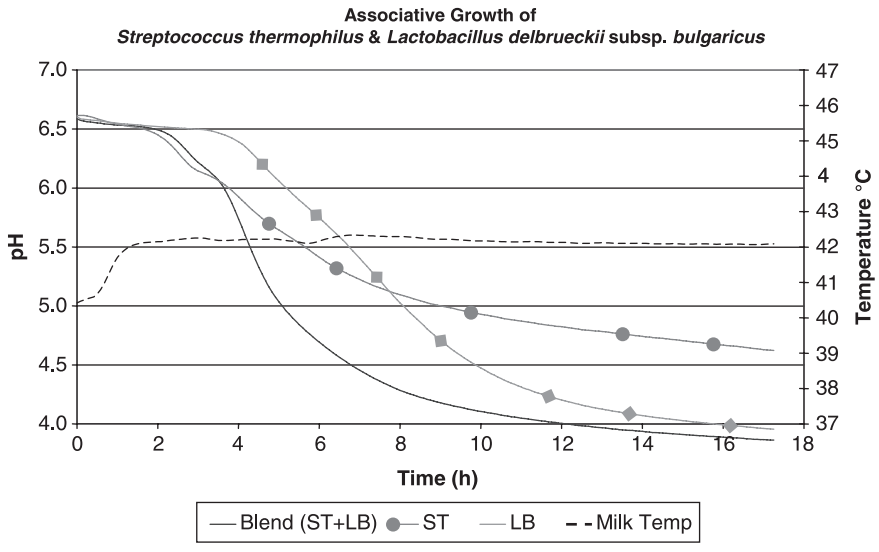


Figure 76.8 *Acidity development in a mixed culture of Streptococcus thermophilus and Lactobacillus bulgaricus. Mixed culture in a 50:50 ratio (solid line), Streptococcus thermophilus alone (circles), Lactobacillus bulgaricus (diamonds), milk temperature (dashed line). Reprinted with permission from Danisco USA Inc.*

Streptococcus thermophilus and *Lactobacillus bulgaricus* exhibit associative growth, the combination of the two leading to faster milk fermentation than either singly, as shown in Figure 76.8 (Davis 1975; Moon and Reinbold 1976; Tamime and Robinson 1999). When the two are fermented together, *Lactobaccillus bulgaricus* starts the fermentation, producing amino acids that promote streptococci growth. *Streptococcus thermophilus* continues the fermentation, rapidly producing lactic acid from lactose to about pH 5.0, after which *Lactobacillus bulgaricus* growth dominates (Tramer 1973). As it grows, *Streptococcus thermophilus* releases substances which promote the growth of *Lactobacillus bulgaricus*. The most important of these are carbon dioxide and formic acid (Tramer 1973; Driessen and others 1982). As growth factors for *Lactobacillus bulgaricus* increase in milk, *Lactobacillus bulgaricus* growth increases. As the pH decreases, *Streptococcus thermophilus* growth diminishes, because *Streptococcus thermophilus* is less acid tolerant than *Lactobacillus bulgaricus*. *Lactobacillus bulgaricus* is more proteolytic than *Streptococcus thermophilus*, and produces amino acids that promote *Streptococcus thermophilus* growth (Bautista and others 1966; Rašić and Kurmann 1978; Tramer 1973). Whereas *Streptococcus thermophilus* is responsible for the bulk of acid production at the start of fermentation, *Lactobacillus bulgaricus* is responsible for the bulk of acid production at the end of fermentation (as well as postfermentation acidification in the finished product during storage) (Kneifel and others 1993; Beal and others 1999). Fermentation rate depends on a balance of fermentation temperature, total solids in the mix, and inoculation rate for any given starter (Kristo and others 2003). The optimum temperature for growth of the two cultures together is 42.5°C (108.5°F) (Mocquot and Hurel 1970).

Starter cultures are chosen on the basis of flavor produced (whether mild or traditional is desired), resistance to phage and antibiotics, rapid acid development in milk, tolerance to sugar, ability to grow in mixed culture, minimal postfermentation acidification, production of exopolysaccharides, adaptability for large scale production, stability in finished product,

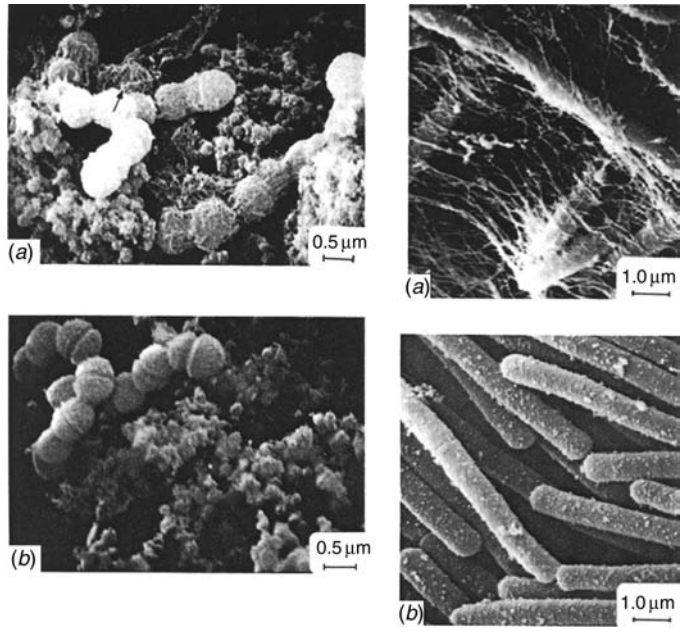


Figure 76.9 *Micrographs of ropy and non-ropy bacteria. Streptococcus thermophilus (left) (ropy top, non-ropy bottom) and Lactobacillus bulgaricus (right) (ropy top, non-ropy bottom). (Schellhaass and Morris 1985). Reprinted with permission from S. Schellhaass.*

stability during cryogenic preservation, frozen storage, and distribution (Mocquot and Hurel 1970; Tramer 1973; Davis 1975; Sellars 1989). Traditionally, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have been used in a rods to cocci ratio that is 1 : 1 to 1 : 15 (Humphreys and Plunkett 1969; Davis 1975; Chandan and Shahani 1995), with one streptococci actually often corresponding to a clump of 5–10 cells (Tamime and Deeth 1980). The ratio used will be primary in determining the final product pH (Torriani and others 1996). Typical specifications for starter cultures are described by Sellars (1989).

Ropy, or exopolysaccharide (EPS) producing strains of yogurt cultures can improve yogurt texture by producing either capsular or filamentous polysachharide exudate (see Fig. 76.9) (Tramer 1973; Davis 1975; Rašić and Kurmann 1978; Schellhaass 1983; Schellhaass and Morris 1985; Hess and others 1997; Duboc and Mollet 2001; Olson 2002). Exopolysaccharides can increase stirred viscosity and decrease syneresis (Schellhaass and Morris 1985; Tamime and Robinson 1999; Duggan and Waghorne 2003). The effect of EPS on texture is less noticeable in stirred yogurts, suggesting that these bonds do not reknit after mechanical stress (Duggan and Waghorne 2003), and is greatest for low solids yogurts (Maiocchi 2003). The type of exopolysaccharide produced, the composition of the milk, the break pH, the growth phase of the organisms and the fermentation temperature all impact the influence of ropy strains on yogurt texture (Duboc and Mollet 2001; Laws and Marshall 2001). Because lower fermentation temperature can favor *Streptococcus thermophilus* which is more commonly "ropy," lower fermentation temperatures can lead to greater exopolysaccharide production (Davis 1975). However, EPS can be produced by both *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Davis 1975).

Acetaldehyde (mainly produced by *Lactobacillus bulgaricus*) and to a lesser extent diacetyl (mainly produced by *Streptococcus thermophilus*) are important to yogurt

flavor (Davis 1975; Rašić and Kurmann 1978). Sugar at levels greater or equal to 8% in the yogurt mix can decrease acetaldehyde production (Davis 1975).

Lactobacillus acidophilus is added to yogurt for health properties, but can exhibit poor survival in yogurt (Sodini and others 2002) due to its acid intolerance (Mital and Garg 1992) and slow growth attributed to lack of proper growth factors in milk (Tramer 1973). It is not advisable to add *Lactobacillus acidophilus* after fermentation; rather *Lactobacillus acidophilus* should be grown with yogurt starter or added to the milk blend at the time of culture addition since it can be inhibited by hydrogen peroxide produced by *Lactobacillus bulgaricus* (Mital and Garg 1992). Growth of *Lactobacillus acidophilus* with yogurt cultures may increase the survival of the acidophilus in yogurt by increasing the hydrogen peroxide tolerance of the organisms (Lourens-Hattingh and Vijoen 2001). Addition of cysteine can improve the survival of *Lactobacillus acidophilus* in yogurt, possibly by increasing the redox potential (Dave and Shah 1997). Selection of the starter culture strains can greatly influence the survivability of adjunct strains such as *Lactobacillus acidophilus* or *bifidobacterium* (Lourens-Hattingh and Vijoen 2001).

Streptococcus thermophilus is easily identified among the lactics by its unique high temperature tolerance. *Lactobacillus bulgaricus* is less easy to distinguish from other

TABLE 76.1 Distinguishing Characteristics.

Characteristic	*Streptococcus salvarius subsp. thermophilus (Streptococcus thermophilus)*	*Lactobacillus delbrueckii subsp. bulgaricus (Lactobacillus bulgaricus)*	*Lactobacillus acidophilus*
Catalase	−	−	−
Gram	+	+	+
Morphology	Small cocci in pairs to long chains (0.7–0.9 μm)	Long, straight sided rods singly, paired, or in short chains (0.5–0.8 × 2–9 μm)	Long, straight sided rods singly, paired, or in chains (0.6–0.9 × 1.5–6 μm)
Atmosphere	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe
pH for growth	6.5 optimum	5.5 optimum	5.5–6.0 optimum
Optimum temp for growth (°C)	40–45	40–45	37
Growth at ≤10°C	−	+ slight	−
Growth at 45°C	+	+	+
Growth at 50–55°C	+	+	−
Heat tolerance (60°C, 30 min)	++	+	−
Fermentation			
Glucose	+	+	+
Fructose	+	+	+
Lactose	+	+	+
Galactose	−	−	+
Sucrose	+	−	+
Maltose	−	−	+
Lactic acid optical rotation	L(+)	D(−)	DL
Acid in milk (%)	0.8	1.5–2.0	0.3–2.0
Fermentation	Homofermentative	Homofermentative	Homofermentative

Source: Kandler and Weiss (1986); Hardie (1986); Davis (1975); Tamime and Robinson (1999); Mocquot and Hurel (1970); Chandan and Shahani (1995); Mital and Garg (1992); Chandan (1982).

lactobacilli and is often confused with *L. lactis*. Distinguishing characteristics of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* are outlined in Table 76.1. Environmental conditions can alter lactic morphology, and the presence of antibiotics, high solids or repeated culture transfers can lead to *Streptococcus thermophilus* that are elongated cocci (Rašić and Kurmann 1978).

Storage time, fermentation temperature, final fermentation pH, yogurt formulation, and strain selection affect the bacterial concentration to be found in the finished yogurt (Beal and others 1999; Kneifel and others 1993). *Streptococcus thermophilus* is the predominant microorganism found during storage (Vinderola and others 2000; Kristo and others 2003; Gueimonde and others 2004). Two weeks after production yogurts typically contain $10^7 - 10^9$ CFU/mL (Kneifel and others 1993).

76.7 YOGURT NUTRITION

Yogurt is a nutritious food that can deliver multiple health benefits to consumers (McKinley 2004). Yogurt, like milk, can be a good source of high quality protein, and important vitamins and minerals. The level of protein and calcium varies in commercial products, depending on the level of milk solids and nutrients that are added above the required minimums. In the United States, whey protein concentrates are often added as a protein source, but these concentrates usually have less calcium and lactose on a protein equivalent basis than skim milk powder.

Fermented dairy products have long been thought to confer special health benefits. Professor Elie Metchnikoff in 1908 (Metchnikoff 1908) reported research being conducted on the use of fermented products by indigenous cultures. He suggested that the cultures in fermented dairy products, especially Bulgarian *bacillus* species, helped to prevent intestinal putrefaction, thereby prolonging life. Research into the health benefits of yogurt have focused on the traditional yogurt cultures, and on adjunct cultures that are often added for their health benefits.

In the United States, the National Yogurt Association (NYA) has developed a program whereby manufacturers of yogurt with live and active cultures are permitted to pay for the right to display a seal on their packaging. The seal allows manufacturers to clearly identify to consumers which yogurts contain active cultures. The use of the symbol requires that the yogurt must contain 10^8 colony forming units at the time of manufacture, and the organisms must be capable of a one log increase in growth after culturing into milk at the end of the product's shelf-life (National Yogurt Association 2004).

Yogurt is generally an excellent source of calcium, a nutrient important for development and maintenance of strong bones and teeth. Recently, a link between weight loss and sufficient calcium intake was established. Consumption of calcium from dairy foods such as yogurt has been shown to contribute more fat loss and better retention of lean body mass during weight loss while following a restricted calorie diet (Zemel 2004; Zemel and others 2004).

Scientific documentation of the benefits of ingestion of yogurt cultures has focused on the benefit to lactose intolerant individuals. During fermentation, the lactose content of milk is reduced by up to 30% (Chandan 1982; Tamime and Robinson 1999; Adolfsson 2004). Approximately 80% of adults in the world have lost the ability to split the β-glycosidic bond in lactose (Barth 1996). This is very much dependent on the race of the individual. Only 20% of caucasians have problems with lactose maldigestion, while

levels approaching 100% of Africans and Asians lack the necessary enzyme (Barth 1996; Adolfsson 2004). When undigested lactose is fermented in the colon uncomfortable symptoms of gas and diarrhea can result. The reduction of lactose by fermentation and the active enzymes released from the yogurt cultures when cells burst have been shown to improve the digestibility of the milk and yogurt for lactose intolerant individuals (Martini and Savaiano 1988; Saavedra and others 2002). Although the two yogurt cultures, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, cannot colonize the human intestine, they have been found to remain viable in the small intestine for 3 to 6 h after yogurt consumption (Robins-Browne and Levine 1981) and can be detected, along with accompanying beta-galactosidase activity, in the duodenum for 1 h after ingestion (Pochart and others 1989). However, Pedrosa and others (1995) could not isolate yogurt cultures from the stomach after ingestion.

In addition to the required yogurt cultures, probiotic cultures are increasingly being added to yogurt. Probiotics are live microorganisms that exert health benefits when ingested in adequate amounts. The most common probiotic in the United States is *Lactobacillus acidophilus* which is added to most yogurts sold in the United States. However, survival of *L. acidophilus* can be poor in fermented milk products, so unless culture counts are declared, the consumer will not know the dose of *L. acidophilus* ingested. Other cultures commonly added to yogurt are *L. acidophilus*, *L. lactis*, *L. casei*, *L. helveticus*, and *Bifidobacterium* species (*longum*, *bifidum*, *infantis*) (Ming and others 1989; Sellars 1989).

Many factors affect the survival of probiotic organisms in yogurt and after ingestion. Probiotic survival is influenced by yogurt composition, oxygen content, and pH (Vinderola and others 2000; Chen and others 2004; Talwalkar and Kailasapathy 2004). Prebiotic ingredients incorporated into the base or coencapsulated with the organisms can enhance survival of some probiotics (Varga and others 2003; Akalin and others 2004; Anjani and others 2004). Screening for organisms that are acid and bile tolerant is essential to ensure that they survive transit through the GI tract and contribute to the intestinal microflora (Saarela and others 2002). Encapsulation can improve survivability of acid and bile sensitive probiotics (Goodward and Kailasapathy 2003; Sridar and others 2003).

Some health benefits that have been postulated for yogurt and probiotics are:

- Lower serum cholesterol
- Stimulate the immune system
- Reduce risk factors for colon cancer
- Reduce lactose intolerance
- Increase calcium absorption
- Synthesize vitamins and predigest proteins
- Normalize the intestinal microflora (i.e., help with irritable bowel syndrome, inflammatory bowel disease, resistance to colonization by pathogens)
- Improve digestive regularity.

Streptococcus thermophilus and *Lactobacillus bulgaricus* have been shown to confer some of the above benefits which are thought to include aiding with lactose intolerance, stimulation of immune function, and reduction in tumor formation (Meydani and Ha 2000; Saavedra and others 2002). There are also other nonbacterial components of

yogurt that have been shown in some studies to stimulate immune function. These include peptides produced during fermentation, cysteine from whey proteins, conjugated linoleic acids and soluble minerals (specifically calcium) (Meydani and Ha 2000).

There are many excellent references summarizing studies of yogurt and probiotic cultures and their effects on health (Chandan 1989; Robinson 1991; Fernandez and others 1992; Havenaar and others 1992; Barth 1996; National Dairy Council 1997; Meydani and Ha 2000; Mattila-Sandholm and others 2003; Adolfsson 2004). A problem with some studies is the inability to derive firm conclusions due to failures in study design that involve controls, administration of the probiotic to the organism, the use of in vitro indicators, and the short duration of treatment (Meydani and Ha 2000). Because of the difficulties in interpreting results and in understanding which organisms are most beneficial, the European Union has undertaken the PROEUHEALTH initiative. The goals of this program are to maintain and build confidence in probiotic products and to improve the health and quality of life of EU consumers. The research institutions involved are working to develop appropriate research tools, understand the mechanisms of probiotic action, investigate the effects on health in humans, and develop the technological properties of the organisms (Saarela and others 2002).

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77

Infant Formulas

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77.1 Introduction 678
77.2 Lipids 679
77.2.1 Long Chain Polyunsaturated Fatty Acids (LCPUFA) 680
77.2.1.1 Preformed Dietary LCPUFA as Components of Biological Membranes 680
77.2.1.2 Physiological Importance of LCPUFA in Infant Formulas 681
77.2.1.3 Side Effects 685
77.2.1.4 Recommendations for the Supplementation of Infant Formulas 685
77.2.2 Gangliosides 686
77.2.3 Analytical Techniques 688
77.3 Carbohydrates 688
77.3.1 Carbohydrate Components that Influence the Intestinal Microflora 689
77.3.1.1 Oligosaccharides as an Important Prebiotic Factor in Human Milk 690
77.3.1.2 Effects of Prebiotic Oligosaccharides of Nonmilk Origin 690
77.3.1.3 Side Effects of Prebiotic Oligosaccharides 691
77.3.1.4 Recommendations for the Supplementation of Infant Formulas with Prebiotic Oligosaccharides 692
77.3.2 Analytical Methods 692
77.3.3 Future Developments 693
77.4 Proteins 693
77.4.1 Human Milk Proteins in Comparison to Bovine Milk Proteins 695
77.4.1.1 Caseins 695
77.4.1.2 Whey Proteins 696

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

77.4.1.3 Bovine Milk Protein Hydrolysates 698
77.4.2 Plant Proteins as Sources for Infant Formulas 698
77.4.2.1 Soy Proteins 698
77.4.2.2 Other Plant Proteins 698
77.4.3 Analytical Techniques 698
77.4.3.1 Whey Proteins 698
77.4.3.2 Caseins 705
77.4.3.3 Milk Protein Hydrolysates 705
77.4.4 Future Trends 705
77.5 Next Steps for Development of Functional Compounds for Infant Formulas 706
References 706

77.1 INTRODUCTION

Human milk is widely accepted as the ideal nutrition for infants: it does supply all necessary nutrients for rapid growth in sufficient amounts, without overloading the functional capacity of the not yet developed gastrointestinal tract or metabolism. This is especially achieved by the high bioavailability of the nutrients, so that relatively low concentrations ensure a sufficient supply (Oddy 2002).

In addition, human milk contains a lot of components, which have a functional importance rather than a nutritive one. In Table 77.1 the most relevant compounds are summarized. However, the list of these components is very long and the specific functions of a lot of these components present in human milk are still not fully understood (Oddy 2002).

When taking growth and development of the breastfed infant as a starting point, instead of considering the composition of human milk, new approaches for infant nutrition arise that could not have been won from the composition of human milk alone. In a lot of respects, breastfed infants develop differently compared to infants with artificial feeding (Davis 2001). Apart from a reduced incidence of allergic or atopic diseases (Garofalo and Goldman 1999; Kelly and Coutts 2000; Halken and Host 2001) as well as a reduced incidence of infections (Hanson and Korotkova 2002), the varying incidence of diabetes mellitus type 1 (Wasmuth and Kolb 2000) or the better cognitive functions of breastfed infants in later life are also worth mentioning (Morley and Lucas 1997). If – compared to breastfed infants – the same capacity for development should be offered to artificially fed infants, functional components have to be found that are able to compensate for the differences still present.

Another important functional aspect is the possibility that in the long term the homeostasis of important metabolic processes can be programmed in infancy by the diet. The aspect of programming homeostasis mechanisms is of ever-growing importance with regard to judging the security of functional components in infant nutrition (Koletzko and others 2002). Theoretically, components newly introduced into infant nutrition might have a negative influence on the process of programming, thus preparing the ground for later diseases, without causing negative side effects during application in infancy.

Out of the many functional components in human milk, some have already gained admission to the practice of infant nutrition. Thus, it has always been a fundamental consideration to use important functional components of human milk in infant formula as well. This is managed in the minor part by isolating such components from other sources than human milk or by searching for alternatively available components, which at a different structure have comparable effects to human milk. In the present chapter, especially lipids and food

TABLE 77.1 Bioactive Components of Human Milk and Their Functions.

Bioacitve Component	Described Function
ACTH and cortisol	Stimulate the maturation of enterocytes and intestinal barrier, modulation of the intestinal immune system
Prolactin	Regulate the maturation of the neuroendocrine and the reproductive system
Gastrointestinal hormones	Influence the function, growth, and maturation of the gastrointestinal tract
Erythropoetin	Stimulates formation of red blood cells
Growth factors IGF, EGF, TGF	Stimulate growth and maturation of the gastro-intestinal tract
Growth factor TGF β	Modulate directly immune system
Growth factor NGF	Influence neuronal maturation and cognitive functions
Cytokines	Infleunces the postnatal development of the immune system
Vitamines A, C, E; prostaglandins, inhibitors of enzymes	Reduce inflammatory reaction
Colony-stimulating factors (CSF)	Stimulate cell growth
Immune globulin sIgA	Defences against pathogens, protects intestinal mucosa
Living cells (macrophages, neutrophils, lymphocytes)	Protect against infections by bacteria and fungi
Nucleotides	Influence intestinal flora, might be involved in postnatal modulation of the immune system
Glutamine	Influences metabolism of enterocytes, possibly protective against infections
Lactoferrine, lactoferricine	Protects against infections by bacteria, viruses, and fungi, antioxidative
Long chain polyunsaturated fatty acids (LCPUFA)	Stimulate neuronal and cognitive development, modulatory effects on immune system and inflammation response
Cholesterol	Supports the enterocytes with cholesterol, has programming effect on cholesterol synthesis, and influences bile acid metabolism
Oligosaccharides	Stimulates the development of a beneficial intestinal flora (prebiotic effect of neutral oligosaccharides), modulate the immune system, and decrease the adhesion of pathogens on the surface of intestinal epithlial cells (mainly acidic oligosaccharides)

components that have an influence on the intestinal flora are reviewed. Although not present in all products, such components are on the market as part of infant formulas.

77.2 LIPIDS

Lipids are the main energy source in breast milk (>50% of energy). At birth, the infant's gastrointestinal tract is immature. In particular, the capacity to digest fat is limited mainly due to a low activity of the pancreatic lipase (Boehm and others 1995a,b) but also due to a low concentration of bile acids in the duodenal juice (Boehm and others 1996a). In breast milk, a bile acid stimulated lipase is present that helps the infant to digest the high amount

of lipids. Additionally, the critical palmitic acid is preferentially in the n-2 position of the lipid which protects the fatty acid for soap formation and subsequently low absorption. In infant formula, the addition of lipase has not been used in mass products but there are some products on the market containing artificial lipids that contain palmitic acid preferentially up to 60%) in the n-2 position (Schmelze and others 2003).

The unsaturated and monosaturated fatty acids up to a chain length of 18 carbon atoms are mainly used as energy source. The polyunsaturated fatty acids are mainly used to be incorporated into biological membranes. Therefore, for functional aspects the long chain polyunsaturated fatty acids are in the focus of the discussion.

77.2.1 Long Chain Polyunsaturated Fatty Acids (LCPUFA)

Long chain polyunsaturated fatty acids (LCPUFA) are important components of all biological membranes in the form of phospholipids. Thus, sufficient availability is an important prerequisite for practically all cell functions. In addition, some LCPUFA serve as precursors for eicosanoids, thereby influencing the immunological development and the inflammatory response of the infant. LCPUFA are defined as fatty acids with more than 18 carbon atoms. Corresponding to the position of the double bindings, one distinguishes between the n-3 and the n-6 LCPUFA-family (Fig. 77.1). Like all animals, human beings have the necessary enzyme system to synthesize these fatty acids from their essential precursors α-linolenic acid (C18 : 3 n-3) and linoleic acid (C18 : 2 n-6). Higher plants only contain fatty acids with a maximum chain length of 18 carbon atoms.

77.2.1.1 Preformed Dietary LCPUFA as Components of Biological Membranes. Human milk contains LCPUFA in relatively high concentrations, quantitatively most important are arachidonic acid (C20 : 4 n-6) and docosahexaenoic acid (C22 : 6 n-3) (Koletzko and others 1992). During intrauterine and postnatal development, there is a considerable accumulation of these two fatty acids in the brain and other tissues.

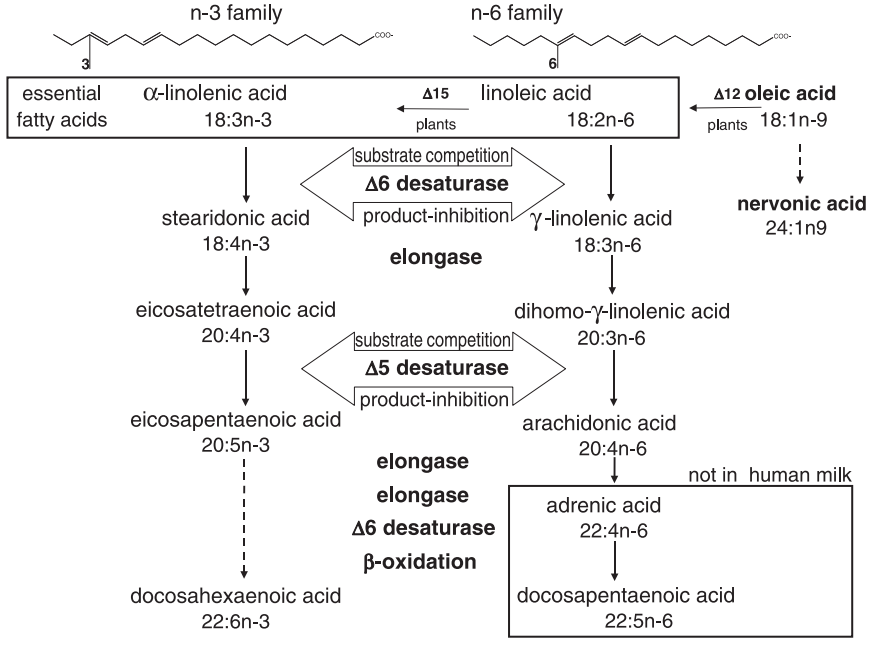

Figure 77.1 *Pathways of the two fatty acid families from the essential precursor fatty acids.*

As a result, the hypothesis arose that in addition to the essential fatty acids α-linolenic acid and linoleic acid, LCPUFA should be supplied with the diet in infancy as semiessential fatty acids. Since the beginning of the 1990s and based on the respective state of knowledge, various committees expressed recommendations for the supplementation of infant formulas with LCPUFA (ESPGAN 1991; BNF 1992; FAO/WHO 1994; ISSFAL 1994; EEC 1996; Koletzko and others 2001; SCF 2003) (Table 77.2).

It has been demonstrated in a lot of studies that in breastfed infants proportionately more LCPUFA were found in plasma lipids as well as in erythrocyte membranes, compared to infants fed a formula without LCPUFA. Besides, the content of essential precursor fatty acids was always sufficient in the respective study formulas. However, if these formulas are supplemented with LCPUFA, comparable fatty acid patterns in plasma lipids or erythrocyte membranes can be achieved as in breastfed infants (Koletzko and others 1995; Makrides and others 1995; Boehm and others 1996b).

When measuring the LCPUFA synthesis by means of [C^{13}] labeled fatty acids, it was clearly shown that both term and preterm infants are already able to synthesize LCPUFA from precursor fatty acids with 18 carbon atoms right after birth (Carnielli and others 1996; Szitanyi and others 1999). The already mentioned examinations of phospholipids in plasma and erythrocytes clearly show that the capacity for synthesis obviously does not match the huge requirements of the rapidly growing organism (Koletzko and others 1995; Makrides and others 1995; Boehm and others 1997b). Recent baboon studies performed with [C^{13}] marked arachidonic acid showed that LCPUFA from milk are actually not transferred to the general carbon cycle, that is, the dietary arachidonic acid was not xoydised, but was preferably used as structural components of biological membranes (Wijendran and others 2002a). From a metabolic point of view, this shows very clearly the importance of preformed LCPUFA for the nutrition during the first months of life.

In Table 77.3 the most important recommendations for the supplementation of infant formula with LCPUFA for preterm and term infants are summarized. They are all based on the assumption that infant formulas should contain LCPUFA at the same concentration as in human milk. Although influences of the maternal diet on the composition of human milk have been described, the pattern of LCPUFA in the lipid fraction of human milk seems to be relatively constant (Koletzko and others 1992; Jensen and others 2000). This is mainly due to the fact that the majority of LCPUFA in human milk derive from maternal stores, while only 10–15% derive from current nutrition (Sauerwald and others 2000).

Human milk contains all n-3 fatty acids up to docosahexaenoic acid and all n-6 fatty acids up to arachidonic acid. There are further fatty acids in the tissue that derive from arachidonic acid by elongation and desaturation (Fig. 77.1). These fatty acids are not present in human milk. Thus, arachidonic acid seems to be an important dietetic precursor for these fatty acids, which are especially important to the myelinization in neuronal tissues (Martinez and Mougan 1998; Wijendran and others 2002).

The present data allow the conclusion that in spite of a sufficient supply with essential fatty acids, preformed dietary LCPUFA from human milk are preferably incorporated into biological membranes. This can be similarly achieved by supplementing formulas with LCPUFA-containing lipids.

77.2.1.2 Physiological Importance of LCPUFA in Infant Formulas

Effect of LCPUFA on Brain Development. The putative significance of LCPUFA for the development of the rapidly growing brain of the newborn was already a matter of

TABLE 77.2 Recommendation of LCPUFA in Infant Milk Formulas for Term and Preterm Infants.

	Term Infants			Preterm Infants		
	n6 LCPUFA	n3 LCPUFA	Ratio n6/r	n6 LCPUFA	n3 LCPUFA	Ratio n6/n3
SCF 2003	LA: 0.5 g/100 kcal formula ARA: 0.35% total of fat	LA: 0.5 g/100 kcal formula ARA: 0.2% total of fat	LA/ALA: 5–15 ARA/DHA: 1.75	–	–	–
	LCPUFA SN-2 associated with phospholipids important; phospholipids content ≤1 g/L liquid formula					
Consensus Konferenz 1999	0.35% ARA/total fat content	0.2% DHA/total fat content	1.75	0.4%/total fat content	0.35%/total fat content	1.14
EU Commission	2% n6 LCPUFA/total fat content	1% n3 LCPUFA/ total fat content	2	–	–	–
WHO/FAO 1995	40 ARA mg/kg body weight	20 DHA mg/kg body weight	2	60 ARA mg/kg body weight	40 DHA mg/kg body weight	1.5
ISSFAL 1994	–	–	–	60–100 ARA mg/kg body weight	70–150 DHA mg/kg body weight	0.85
BNF 1992	2–1% n6 LCPUFA/ total fat content	1–0.5% n3 LCPUFA/total fat content	2	–	–	–
ESPGAN 1991	2–1% n6 LCPUFA/ total fat content	1–0.5% n3 LCPUFA/total fat content	2	1% n6 LCPUFA/total fat content	0.5% n3 LCPUFA/ total fat content	2

TABLE 77.3 Most Important Nonmilk Oligosacchrides Already Used in Infant Milk Formulas.

Prebiotic Ingredient	Source
Galacto-oligosacchrides	Enzymatic synthesis from lactose
Fructo-oligosacchrides (inulin-type)	Extraction from plants
	Enzymatic hydrolysis from natural polymers
	Enzymatic synthesis from suchrose
Palatinose/isomaltulose-oligosaccharides	Enzymatic synthesis from suchrose
Soya-oligosaccharides	Extraction from soya
Lactosucrose	Enzymatic synthesis from lactose
Xylo-oligosaccharides	Enzymatic hydrolysis from natual polymers

interest at an early stage, since the neuronal tissue is very rich in LCPUFA (Martinez and Mougan 1998). This was supported by the investigations of the study group of A. Lucas, which very early pointed to the significance of nutrition for brain development (Lucas and others 1992). Additionally it could be shown to show that the fatty acid composition of erythrocytes is concomitant to that of the brain indicating that the measurements of fatty acid pattern of red blood cell membrane are relevant also for the neuronal tissue (Makrides and others 1994). As a consequence, breastfeeding is associated with improved neurological conditions (Bouwstra and others 2003b) as well as cognitive functions (Hadders-Algra 2005). Based on these data it is widely accepted that a supplementation of infant formulas could support the development of the brain.

Infant formulas that are supplemented with LCPUFA are commercially available in Europe since 1992. However, the debate about a possible benefit of LCPUFA in infant formula is not over yet. There is only a fairly good consensus that preterm formulas should contain LCPUFA. The attitude towards the benefit of LCPUFA for healthy term infants is by far less unanimous. The most important reason for that it is difficult to measure brain function. Internationally recognized neurological tests have been developed, in order to differentiate between a normal development from a disturbed one. Due to the enormous ability of the brain to compensate for harmful effects during development (so-called "plasticity" of the brain (Beaulieu 2002), it is, however, very difficult to prove positive effects of a supplementation with LCPUFA during the first months of life with these methods at a later age as well (Koletzko and others 2001; Bouwstra and others 2005). Thus, first studies have been started to develop new neurological tests, which are able to measure differences of the development within the normal variations.

Recently, it was found that the fatty acid status at birth has an important impact on the postnatal development of brain functions indicating that the fatty acid supply to the pregnant woman is also important. High intake of trans fatty acids was accompanied by lower levels of docosahexaenoic and arachidonic acid in the infant's tissue (Desci and others 2002). The fatty acid status at birth had significant influence on the neurological behavior immediately after birth (Dijck-Brouwer and others 2005), on general movement quality at three months of age (Bouwstra and others 2003a, 2006a) and on cognitive functions at 18 months of age (Bouwstra and others 2006b).

Since still many aspects of the LCPUFA metabolism are under investigation, there are no final statements with regard to the necessity of a supplementation of infant formulas for

healthy term infants. However, there is increasing evidence that LCPUFA are helpful for supporting brain development during the first months of life (Khedr and others 2004; Hadders-Algra 2005).

Effect of LCPUFA on the Development of the Immune System. LCPUFA are an important link between fat metabolism and the immune system. On the one hand, they are precursors of important mediator-substances, the so-called eicosanoids, which play an important role in inflammations (Calder and Grimble 2002; Calder 2003). The most important fatty acids, which serve as precursors for eicosanoids are eicosapentaenoic acid (C20 : 5 n-3), docosahexaenoic acid (C22 : 6 n-3), dihomo-γ-linolenic acid (C20 : 3 n-6), and arachidonic acid (C20 : 4 n-6). On the other hand, some LCPUFA regulate the formation of eicosanoids from their respective precursors (Guichardant and others 1993; Keichler and others 1995; Ringbom and others 2001) by inhibition of the enzymes cyclooxidase and lipoxigenase indicating a very crosslinked regulative system which can be influenced by dietetic measures (Kelley and others 1997; Calder 1999). While there is considerable, clinically relevant evidence for older children and adults regarding the dietetic application of specified lipid mixtures in chronic-inflammatory diseases (Suter 1994; James and Cleland 1997; Belluzi and Miglio 1998; Ziboh 1998), there are practically no studies in newborns. This is partly based on the LCPUFA requirements of the newborn, which are essentially determined by the requirements for growth. In addition, the specific constellation of the immune system of newborns does not allow a direct comparison to the immune systems of adults with regard to the effect of fatty acids. At birth, the immune system is dominated by Th2-reactions. This is necessary until the end of pregnancy, in order to suppress cytological reactions of the Th1-type, which would result in an immunological conflict between mother and fetus (Langrish and others 2002; Morein and others 2002).

However, there are recent studies showing that there is a relationship between LCPUFA supply during infancy and postnatal development of the immune system. The fatty acid profile of the milk of atopic mothers is characterized by relatively low concentrations of important LCPUFA. This is also reflected in the fatty acid status of the newborn (Beck and others 2000; Kankaanpaa and others 2001). If the human milk contains relatively high amounts of n-6 and low amounts of n-3 fatty acids, breastfed infants also show atopic symptoms even during the first year of life (Duchen and Björksen 2001). Field and others (2000) were able to show that by supplementing an infant formula with LCPUFA, the lymphocyte population and cytokine production were comparable to breastfed infants.

It is known from animal studies that LCPUFA can promote the growth of the thymus gland (Guimaraes and Curi 1991; Fernandez and others 1997). Thus, LCPUFA are directly linked to the postnatal development of a central organ of the immune system. So far, there are no systematic studies in human newborns on this subject. However, it was shown by sonographic measurement of the thymus size that in breastfed infants during the first three months of life, the thymus tissue growths much better than in infants fed formula without LCPUFA (Hasselbalch and others 1999). Results of studies showing in how far the difference between breastfeed and formula fed infants can be corrected by LCPUFA supplementation are not available yet.

In summary, it may be said that LCPUFA in human milk have an important immunomodulating task. Therefore, the supplementation of infant formulas with LCPUFA should not exclusively be considered on the basis of structures and function of membranes of the brain.

Further Physiological Effects of LCPUFA. Due to the various effects of eicosanoids, which are not only limited to the immune system, and the far-reaching importance of LCPUFA for the function of biological membranes, impacts on a variety of physiological processes are possible.

Thus, it was reported that the blood pressure in 6-year-olds, who where fed an LCPUFA supplemented infant formula for 3 months as infants, was significantly lower compared to infants fed a formula without LCPUFA (Forsyth and others 2003). This could be of considerable importance with regard to the development of cardiovascular diseases later in life.

Experimental results are plausible with regard to the influence of LCPUFA on the development of bone tissue (Bodin and others 2003). Clinical studies in infants on this subject are not yet available.

As it is too early for a clinical evaluation of such results, they have not been considered yet in the production of fat blends for infant formulas. The results also show that with the progressive state of knowledge new applications are possible.

77.2.1.3 Side Effects. *In vitro* and animal experiments have shown that apoptosis was induced by a high concentration of eicosapentaenoic acid (Heilmi and others 2001; Sweeney and others 2001), while high doses of arachidonic acid led to a reduction in thrombocyte aggregation.

Due to the sensibility of LCPUFA towards oxidation, it was examined whether an additional dose of LCPUFA would have an impact on the peroxidation of lipids. This was not the case (Stier and others 2001).

LCPUFA-supplemented formulas are on the market since 1992 and with regard to the concentrations all manufacturers comply with the recommendations. There have been no reports so far about direct side effects in this concentration range. Therefore, there has been no safety advice accompanying the recommendations (ESPGAN 1991; BNF 1992; FAO/WHO 1994; ISSFAL 1994; EEC 1996; Koletzko and others 2001; SCF 2003).

77.2.1.4 Recommendations for the Supplementation of Infant Formulas. With extracts of egg lipids, the whole range of LCPUFA in form of phospholipids is available as a raw material. Fish oils can be used as an excellent source for all n-3 LCPUFA. Arachidonic acid and docosahexaenoic acid are available in form of triglycerides as so-called single-cell oils. These raw materials have already been used in infant formulas in Europe and the United States in various mixtures. Thus, the technological requirements exist, in order to manufacture every possible combination with regard to the fatty acid profile as well as the form of the lipids.

All LCPUFA in human milk are assigned to have a physiological role. Therefore, one has to assume that a well-balanced ratio of the individual fatty acids is important for the supplementation of infant formulas (SCF 2003).

In all published recommendations so far, the absolute amount of the single LCPUFA but not the form of lipids in which they appear played an important role. However, recent animal studies have shown that the form of the lipid influences the efficiency of the incorporation of LCUFA from milk into structural lipids. For instance, LCPUFA that are offered as phospholipids are incorporated in a significantly more efficient way into lipids, compared to LCPUFA that are administered in the form of triglycerides (Wijendran and others 2002a). This can be partly explained by the fact that LCPUFA from phospholipids are more efficiently absorbed compared to those from triglycerides (Boehm and others 1997; Carnielli and others 1998). In human milk, LCPUFA are

practically not available as free fatty acids, but can be found as 85% triglycerides and 15% phospholipids (Jensen 1996). Due to the influence of the lipid form on the bioavailability of LCPUFA, they should ideally be supplemented in infant formulas as in human milk consisting of both, triglycerides and phospholipids.

The necessary duration of a supplementation is still an unsettled issue. There are no systematically conducted clinical trials with formula-fed infants. Investigations with healthy term infants have shown that with regard to the neurological development, a breastfeeding period of more than six weeks does offer advantages compared to a shorter duration. As the LCPFUA supplementation follows the "ideal" human milk as far as possible, a duration corresponding to the breastfeeding recommendations seems to be useful.

77.2.2 Gangliosides

Gangliosides are a group of sialic acid containing glycosphingolipids. They consist of a lipophilic ceramide moiety and a hydrophilic oligosaccharide chain. Ceramide is comprised of a sphingoid base and an amide bond fatty acid. The oligosaccharide chain is linked to the sphingoid base at the hydroxyl-group at carbon atom 1 (Fig. 77.2).

Gangliosides are vital components of neural membranes first described in ganglia cells by Klenk in 1935 (Klenk 1935). Since then gangliosides could be detected in a diversity of other tissues.

Gangliosides in human milk are part of the membrane fraction of the milk fat globule (Jensen 1996).

The quantitatively most important milk gangliosides are the disialogangloside GD_3 and the monosialoganglioside GM_3 (Huang 1973; Hauttecoeur and others 1985). Moreover, the ganglioside composition of human and bovine milk was shown to be different. While GD_3 is the main ganglioside in bovine milk, GM_3 dominates in human mature milk. The fatty acid composition of GM3 and GD3 differs between human and bovine milk (Bode and others 2004). Because bovine milk is the main basis for infant formula attempts have been made to chance the fatty acids composition of bovine gangliosides toward the composition found in breast milk (Beermann and others 2005).

Human milk possesses a specific pattern of gangliosides dependent on the state of maturation. Bovine milk based IMF ganglioside levels are lower. However, butter milk is a interesting source of milk born gangliosides. Approximately 1–2% of the total lipid content of bovine buttermilk are gangliosides. In human colostrum GD_3 is the main ganglioside whereas in mature human milk GM_3 and several highly polar gangliosides are predominant, dependent on the maturation status (Rueda and others 1998). In addition the fatty acid composition of gangliosides is species specific. In bovine milk, it has been shown that the fatty acid pattern of triacylglycerols, phospholipids and gangliosides depends on the stage of lactation and may be an adjustment to specific metabolic demands (Martin and others 2001). A critical analysis showed that infants fed starter formulas have only 20% ganglioside sialic acid intake of those fed human colostrum or transitional milk. Follow on formulas (4–5 months) provide more or less the same amount ganglioside (also total sialic acids) as mature human milk. A supplementation with sialic acid containing glycoconjugates of infants especially for the first days after delivery is recommended (Sanchez-Diaz and others 1997).

As sialic acid is an important component of the brain tissue, gangliosides have been considered essential for CNS growth and development since many years. There

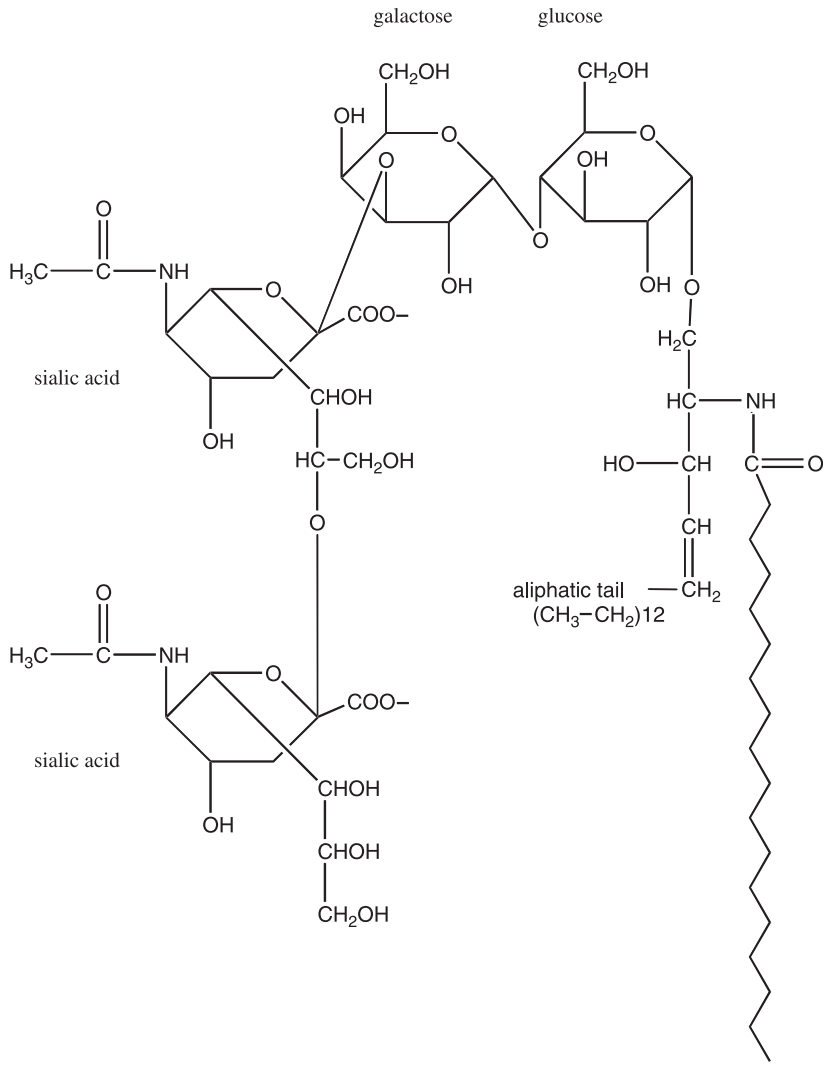

Figure 77.2 *Chemical structure of gangliosides (example: GD_3).*

is evidence that gangliosides are essential for synaptogenesis, and neurotransmission, brain growth and maturation (Wang and others 2003). Human milk is very rich source of sialic acid relative to infant formulas and breast fed infants have two-fold higher concentration of salivary sialic acid. It is conceivable that documented developmental advantages of breastfeeding might be partly explained by higher ganglioside content in brain gray matter, which resulted from a higher intake of exogenous sialic acid like in human milk (Mendez-Otero and Santiago 2003; Khedr and others 2004). Additionally it could be demonstrated that a strong correlation exists between the LCPUFA and ganglioside metabolism of the brain (Wang and others 2003). Consequently, a combination of both ingredients could be a further step of improvement of infant formulas.

77.2.3 Analytical Techniques

Although not completely understood, there is a strong relationship between the biological functions and the molecular structure of lipids. This relation between physiological function and molecular structure of milk lipids efforts an analysis strategy with a systematic process implementing target specific extraction, chromatographic separation and fractionation techniques up to mass-spectrometry methods for molecular structural characterization.

Human or animal milk as a native matrice contain a complex composition of lipids with different polarities and a broad range of different types of integrated fatty acids.

For the structural/qualitative and quantitative analysis extraction methods focusing on the main lipid classes neutral lipids and polar lipids are helpful. Aside this, a rapid method for the determination of total fat in milk products using a supercritical fluid extraction has been developed (Wolf and others 2003).

To separate lipids into the different polarity classes varying in polarity from cholesterolesters up to lysophosphatidylcholine a HPLC method with a triple gradient (hexane, isopropanol, water) with a polyvinyl-alcohol diol/reversephase column techniques is the next step.

This technique is also suitable for the quantitative analysis of major lipid classes. For the optimal quantification of lipophilic molecules and lipid species of the different polarity classes, independent of absorption properties of the eluent and probes and without any derivatization labeling an evaporative light scattering detection is used. With reference to standard calibrations it is possible to quantify separated lipid components derived from milk and other native matrices.

Further neutral- and glycolipids could be separated via HPCL with a binary gradient (acetonitrile, isopropanol) and reverse phase (C18) column technique. Concerning glycolipid-extrations analysis a binary gradient (acetonitrile, phosphate buffer) with a aminopropyl anion-exchange technique is available. The combination of this HPLC lipid class separation combined with an automatic fractionation and completed with gaschromatographic analysis or mass spectrometry allows a rapid and complete lipid structure explanation (Sebedio and Pevkins 1995).

Depending on the question it is possible to analyze the sampled fractions from HPCL separation on fatty acid composition via methyl ester derivatisation and gaschromatography. And using mass spectrometry often coupled with gas or liquid chromatography the exact molecular structure deduced from the molecular mass could be characterized.

The combination of different extraction-methods, chromatography and mass-spectrometry techniques can be used for the determination of the lipid composition of a great variety of source materials and to describe the molecular structure of isolated components.

77.3 CARBOHYDRATES

The main carbohydrate in breast milk is lactose and consequently, the majority of infant formulae contain lactose. Lactose is an easy digestible source of energy. Although the lactase activity of the brush border membrane appears relatively low most infants tolerate lactose well. The tolerance is less developed in Asian populations.

Apart from lactose, breastmilk contains considerable amounts of so called oligosaccharides (OS). These OS are not digestible and have several biological functions (Boehm and Stahl 2003a). Since 1980 OS are defined as carbohydrates with a degree of polymerization up to 10. However, as there is no physiological reason for this definition,

oligosaccharides have been variously defined later on ranging from a degree of polymerization of 2 up to 20 and more (BNF 1990; Kunz and others 2000). Recently the IUB-IUPAC Joint Commission on Biochemical Nomenclature stated that the borderline between oligo- and polysaccharides cannot be drawn strictly. However the term oligosaccharide is commonly used to refer to defined structures as opposed to a polymer of unspecified length (IUPAC and IUBMB 1997).

Free oligosaccharides are naturally a constituent of all placental mammals' milks and can also be found in bacteria, fungi, plants, and so on. Further they derive from hydrolysis of dietary polymers during digestion. Technologically, they can be extracted from natural sources, can be synthesized from monomers and/or small oligosaccharides or derived from hydrolysis of natural polymers.

Human milk contains up to >1 g OS/100 mL indicating that, at least during postnatal development, they play an important physiological role. It is widely accepted that human milk oligosaccharides contribute to the establishment of a particular intestinal flora (dominated by bifidobacteria), the postnatal stimulation of the immune system, to the defence effect of human milk against viral and bacterial infections, and the enhancement of the bioavailability of minerals. Although human milk is the specific diet during the postnatal period, there is evidence that effects of oligosaccharides observed during the postnatal period can also be found in later life.

77.3.1 Carbohydrate Components that Influence the Intestinal Microflora

The intestinal flora of the infant is developed in a stepwise process after birth, which can be influenced by many factors. Modus of birth, extrinsic factors such as medication or nutrition, but also bacterial interactions lead to a very complex flora with about 400 species within the first few weeks of life (Orrhage and Nord 1999). Breastfed infants develop an intestinal flora in which bifidobacteria dominate, while formula-fed infants develop very quickly an intestinal flora comparable to that later in life without a dominating bifidus flora (Harmsen and others 2000).

The physiological significance of the intestinal flora for the host has been the focus of many scientific studies in the past few years. The intestinal flora reacts with the intestinal epithelial cells as well as with the immune system (Köhler and others 2003). The influence of the postnatal development of the immune system is of considerable importance for infancy. First results come to the conclusion that there are tight relations between the intestinal flora during infancy and the development of atopic or allergic diseases later in life (Böttcher and others 2000; Björksen and others 2001; Kalliomaki and others 2001; Woodcock and others 2002). During the past few years, allergic diseases have increased considerably in developed countries. Therefore, there is a huge scientific interest in the possibility to reduce the frequency and severity of such diseases by positively influencing the intestinal flora.

There are basically two concepts to selectively change the intestinal flora by dietetic interventions (Saavedra and Tscherina 2002): living bacteria (so-called probiotic bacteria) are administered into the gastrointestinal tract or the diet is supplemented with components (so-called prebiotics) that positively influence the intestinal flora by selectively promoting specific bacteria. When taking the effects of human milk on the intestinal flora of the infant as an example, the prebiotic way can be considered as the natural way to develop the intestinal flora.

77.3.1.1 Oligosaccharides as an Important Prebiotic Factor in Human Milk. The bifidogenic effect of human milk is known since more than 100 years (Tissier 1900). Various bifidogenic substances have been identified in human milk. Oligosaccharides have been recognized as an especially effective prebiotic in human milk (Newburg 2000).

Oligosaccharides appear in human milk at a concentration of up to >1 g/100 mL, while they are virtually absent in infant formulas. The structure of oligosaccharides in human milk is very complex (Boehm and Stahl 2003) and the functional consequences of these very different structures remain to be elucidated.

Oligosaccharides in human milk are virtually resistant to digestion (Engfer and others 2000; Moro and others 2005). Therefore, they reach the colon, where they can develop their prebiotic effect. Thus, the nondigestibility during the passage through the small intestine is a prerequisite for a prebiotic effect. Structural requirement for the resistance towards digestive enzymes is the beta-glycosidic bonding of galactose in the basic molecule (Fig. 77.3). In fact, the digestive enzymes of the human gastrointestinal tract are only able to split off galactose from an alpha-glycosidic bonding. In contrast, the intestinal bacteria have the enzymes available to utilise these compouds.

77.3.1.2 Effects of Prebiotic Oligosaccharides of Nonmilk Origin. Due to the complexity of OS in human milk and the lack of natural raw materials with identical structure, it was necessary to search for alternative structures with prebiotic effect for the application in infant formulas.

When searching for alternative substances, criteria such as availability, safety of the source and biological efficacy play an important role.

Several oligosaccharides from different sources have been identified as prebiotic components. The most relevant compounds for the use in infant nutrtion are summarized in Table 77.3.

The most extensive experience is available for fructo-oligosaccharides gained from chicory extract and galacto-oligosaccharides gained from enzymatic synthesis of lactose. Initially used for adults, they are the basis for the prebiotic concept for infant formulas since the mid-1990s (Gibson and Roberfroid 1995; Boehm and others 2003).

So far, especially fructo-oligosaccharides and galacto-oligosaccharides were used in infant formulas. The most intensive studies exist for fructans and, to a lesser extent, for

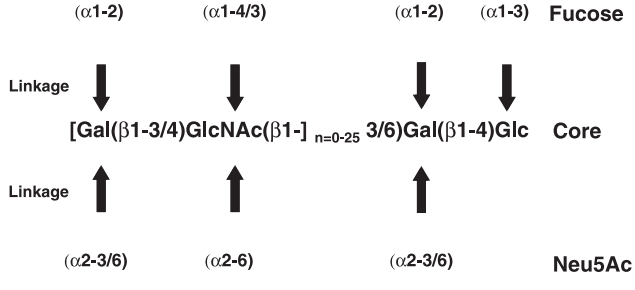

Figure 77.3 *Basic structure of human milk oligosaccharides. The arrows mark the possible location of the glycosidic linkages of the respective fucose and sialic acid residues. Please note that not all possible linkages might occur in a distinct oligosaccharide.*

galactooligosaccharides (Loo and others 1999). Fructans are linear or branched fructose-polymers, which are either mainly β2-1-linked inulins or mainly β2-6-linked levans. Because the inulin type fructans can be easily extracted from plant sources (e.g., asparagus, garlic, leek, onion, artichoke, chicory roots, etc.) they have been widely used as ingredients of dietary products.

Galactooligosaccharides are synthesized from lactose via enzymatic transgalactosylation using β-galactosidases mainly of bacterial origin (e.g., *Bacillus circulans*) (Tanaka and Matsumoto 1998). The commercial use of β-galactosidase in food processing is extensively reviewed elsewhere (Kinsella and Taylor 1995) Galacto-oligosaccharides consists of a chain of galactose molecules with mainly a glucose molecule at the reducing terminus, varying in chain length (degree of polymerization range 3–8) and linkages.

A mixture of these two oligosaccharides has been proven to be particularly effective. The bifidogenic effect was shown in formula-fed preterm (Boehm and others 2002) and term (Moro and others 2002; Schmelze and others 2003) infants. The effect depends on the dosage. At a concentration of 0.8 g/dL (this corresponds to the concentration of neutral oligosaccharides in human milk), the number of bifido bacteria was tantamount to that found in the feces of breastfed infants. The bifidogenic effect was associated with a reduction of the stool Ph (Moro and others 2002) as well as a reduction of pathogenic bacteria (Knol and others 2005a).

Short-chain fatty acids are of considerable importance to the physiological effect of the intestinal flora. They are the fermentation product of bacteria in the colon and are therefore an important characteristic feature of the intestinal flora (Siguur and others 1993). Compared to formula-fed infants, the profile of short-chain fatty acids differs considerably from that of breastfed infants. On supplementing an infant formula with a mixture of galacto-oligosaccharides and inulin, there was a pattern of short-chain fatty acids in feces that corresponded to the pattern found in the faeces of breastfed infants (Knol and others 2005b). The short chain fatty acid pattern reflect the metabolic activity of the entire flora.

Thus, the similarity of fecal short chain fatty acids between breastfed infants and infants fed a formula supplemented with galactooligosaccharides and inulin indicate that the given prebiotic mixture stimulate the entire flora towards the flora of breastfed infants (Boehm and others 2004a).

There is accumulating evidence that the bacteria stimulated by the prebiotic ingredients as well as by their metabolites play an important role in the postnatal developemnt of the immune system (Boehm and others 2004b). The effect could be proven in animal experiments. Feeding galactooligosaccharides and inulin as mixture to mouse a significant stimulation of the cellular (e.g., Th1/Th2) immune balance and reduced the allergic inflammation after antigen exposure (Boehm and others 2004b).

In summary, the conclusion can be drawn that such prebiotic oligosaccharides are able to positively influence the immunological development (Boehm and others 2005).

77.3.1.3 Side Effects of Prebiotic Oligosaccharides. There are no known side effects when applying up to 1 g/100 mL of galacto-oligosaccharides. In theory, higher dosages could display osmotic effects. In clinical trials, concentrations higher than 1 g/100 mL have not been applied. Therefore, such side effects have not been described in infants.

At higher concentrations (>0.5 g/100 mL) fructo-oligosaccharides can cause flatulence. This applies especially to short-chain fructo-oligosaccharides (chain length of up to 10 monomers). As a consequence, some commercially available infant formulas with prebiotic oligosaccharides use especially inulin with a chain length of more than 10 monomers at a relatively low concentration. The Scientific Committee on Food of the EU did not have any safety concerns with regard to a total concentration of 0.8 g/100 mL and a mixture of 90% galacto-oligosaccharides with 10% long-chain fructo-oligosaccharides (ECSCF 2001).

77.3.1.4 Recommendations for the Supplementation of Infant Formulas with Prebiotic Oligosaccharides. Provided that alternative oligosaccharides are similarly effective as neutral oligosaccharides in human milk, the concentration range of supplemented oligosaccharides should be between 0.4–0.8 g/100 mL. This is supported by the results of clinical studies: at a concentration of 0.8 g/100 mL of a galacto-oligosaccharide/fructo-oligosaccharide mixture, the concentration of bifidobacteria in feces was similar to that of breastfed infants (Moro and others 2002; Schmelze 2003).

The effect of prebiotic oligosaccharides depends on a constant supply. Therefore, the duration of the supplementation should follow the recommendations for breastfeeding.

77.3.2 Analytical Methods

Depending on the type, size and structure, as well as the (biological) source of oligosaccharides a variety of separation techniques and methods have to be applied for the characterization of the molecules of interest. The milk oligosaccharides are described in detail by Newburg and Neubauer (1995) and Kunz and others (2000).

For general analytical techniques and methods in the field of glycobiology the reader is referred to Lennarz and Hart (1994) and El Rassi (1995).

Briefly, the major techniques for the analyses of glycans (pure carbohydrates) and glycoconjugates (such as glycoproteins and glycolipids) are chromatography, mass spectrometry (MS), spectroscopy, electrophoretic methods and separation techniques like crystallization, and filtration. Chromatography plays the major role in separation and analyses of oligosaccharides. Besides ion exchange chromatography, affinity chromatography (by lectins or antibodies) and gel permeation GPC, high performance liquid chromatography (HPLC) is the most frequently used technique. As stationary phases normal phase (e.g., amino phases), reversed phases (e.g., C-18) as well as graphitized carbon columns are used. Refractive index, UV and fluorescence, and radioactivity are used as detection principles with evaporative light scattering detection as a technique with a great potential. High pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is a powerful method widely used for carbohydrate analysis (Lee 1996).

For structural analyses of distinct oligosacharides nuclear magnetic resonance spectroscopy (NMR) is a very powerful method but if only minute amounts of samples are available, mass spectrometry plays an important role for sequencing of molecules. Methylation analyses and GC-MS of monosaccharide compounds are widely used for determination of structural features of oligosaccharides. Fast atom bombardement (FAB-MS) enable the structural analyses of mainly derivatized oligosaccharides and more recent techniques like matrix assisted laser desorption/ionization mass spectrometry

(MALDI-MS) and (nano) electrospray ionization mass spectrometry enable the characterization of native (underivatized) carbohydrates. MALDI-MS of glycans and glycoconjugates is excellently reviewed by Harvey (1999). Nano-ESI-MS is especially well suited for analyses of oligosaccharides, glycoproteins, and other glycoconjugates (Karas and others 2000).

As an alternative to PSD MALDI the negative ion nano-ESI-ion trap MS of native underivatized oligosaccharides is even a more powerful technique, since in the MS^n-mode of fragmentation the real sequence from the reducing end of the molecules can be generated with additional information on position of the glycosidic linkage of the released fragment (Pfenninger and others 2002a). The use of MALDI-Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICRMS) offers a direct detection of the distinct unambiguous composition of human milk oligosaccharides by high resolution measurements (Pfenninger and others 2002b).

Especially for the widely used prebiotic oligosaccharides the classical dietary fiber analyses have been insufficient since these molecules show good solubility compared to typical fibers the so called nonstarch polysaccharides. For the analyses of prebiotics like fructans (oligofructoses, inulin) and galactooligosaccharides, AOAC methods (Association of Official Analytical Chemists) have been recently published.

77.3.3 Future Developments

Although the understanding of the molecular mechanisms of dietary oligosaccharides is not complete there are some health benefits which can be directly attributed to these ingredients. This stimulates the future attempts to investigate the structural basis of these functions. In fact, there are some possibilities to produce a particular "designed" oligosaccharide molecules. Therefore, the development of test systems to investigate biological effects of oligosaccharides *in vitro* as well as *in vivo* has a high priority.

Because the possible health benefits of dietary oligosaccharides are species dependent animal studies have a limited importancein elucidating the beneficial effects of oligosaccharides to humans.

The intestinal microbiota plays a key role as a mediator of the physiological effects of dietary oligosaccharides. During the last decade, an enormous progress has been made in improving the techniques in analyzing of fecal microbiota. The intestinal microbiota in humans can usually be studied using fecal samples which does not adequatably reflect the intestinal microbial ecosystem. Therefore, noninvasive methods will be developed to investigate the metabolic activity of the intestinal microbiota using stable isotope techniques.

In summary, dietary oligosaccharides of different origins offer a variety of functional effects which might have significant benefits to the general health of humans. Because oligosaccharides are safe, quite stable and can easily be processed in dairy and food industry there is a great pontential in contributing as functional dietary ingredients in various functional products.

77.4 PROTEINS

Human milk contains a lot of different proteins needed as a well-balanced amino acid source for rapidly growing infants. Therefore, traditionally the quality of a dietary protein for infants is mainly evaluated on the basis of its amino acid composition

TABLE 77.4 Amino Acid Composition of Human Milk.

	Polberger (g/100 g aa)	Picone (g/100 g aa)	Hanning (g/100 g aa)	Räihä (g/100 g aa)	Heine (g/100 g aa)	Volz (g/100 g aa)	Souci (g/100 g aa)	Average (g/100 g aa)
Asp	9.5	9.3	10.0	9.0	8.3	8.6	8.8	9.1
Glu	16.4	18.2	18.5	15.5	17.8	17.0	16.8	17.2
Ser	4.8	4.4	4.7	4.4	5.1	4.4	4.5	4.6
His	2.4	2.5	2.4	2.7	2.3	2.4	2.4	2.4
Gly	2.6	2.4	2.6	2.3	2.6	2.6	2.8	2.6
Thr	4.7	4.6	4.6	5.1	4.6	4.6	4.8	4.7
Arg	3.8	4.0	3.8	4.3	4.0	4.4	3.9	4.0
Ala	4.0	3.9	4.1	3.6	4.0	3.6	4.3	3.9
Tyr	4.5	4.9	4.3	4.4	4.7	5.3	4.3	4.6
Val	5.5	5.5	5.7	6.5	6.0	6.4	6.2	6.0
Met	2.1	1.8	1.3	1.6	1.8	2.2	1.8	1.8
Ile	5.5	5.5	4.9	6.0	5.8	5.6	5.9	5.6
Phe	4.0	4.4	4.0	4.3	4.4	4.6	4.2	4.3
Leu	10.5	12.0	10.0	9.6	10.1	9.5	10.2	10.3
Lys	7.2	5.0	7.1	7.8	6.2	6.9	6.6	6.7
Pro	8.1	8.1	8.2	8.8	8.6	8.3	8.8	8.4
Cys	2.3	1.9	2.0	2.1	1.7	1.9	1.8	2.0
Trp	2.0	1.6	1.6	1.9	1.8	1.8	1.7	1.8
Sum	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

aa: Amino acids.

(Volz and others 1983; Picone and others 1989; Polberger and others 1990; Hanning and others 1992; Lönnerdahl 2003). Although human milk is considered as a "gold standard" for the composition of infant formulas, the milk composition is far away being a standard. In Table 77.4 literature data commonly used as reference are summarized. The variations found in the different studies are the reason that authorities like FDA or EFSA have not similar reference values leading to different quality evaluation of dietary proteins of identical composition.

From a metabolic point of view, the amino acid composition is only one factor of the nutritive quality of a dietary protein. Measurements of postprandial plasma amino acid profiles demonstrate that the postprandial metabolic response differs significantly between human milk protein and bovine milk protein feeding which cannot predict from the composition (Moro and others 1999). The glycosylation of some proteins have been identified as one factor influencing the digestibility but also technological processes such as heating can influence the postprandial amino acid pattern (Boehm and Räihä 1994).

Besides these nutritional proteins also functional proteins were found in human milk (Table 77.5). In the combination of "nutritive" and "functional" proteins, human milk provides an adequate nutrition and protect the infants against infections and facilitate an optimal development of important physiologic functions in newborns (Lönnerdahl 2003).

77.4.1 Human Milk Proteins in Comparison to Bovine Milk Proteins

The most infant milk formulas contain cow's milk proteins, intact or for hypoallergenic formulas in the form of peptides. These proteins show considerable differences to human milk proteins (Table 77.6). The total protein content is three times higher in bovine milk than in human milk and the casein/whey protein ratio is also completely different with approximately 40 : 60 in human milk and 80 : 20 in bovine milk (Prentice 1996). Also the individual proteins belonging to caseins or whey proteins are very different between human milk and bovine milk. The main casein of bovine milk is α-casein which is not found in human milk or only in small traces. The main difference in whey proteins is the high amount of β-lactoglobulin in bovine milk which is missing in human milk (Table 77.6).

Despite these big differences in both milk proteins, the casein whey protein ratio and the amino acid composition of infant milk formulas could be adapted very similar to human milk proteins, which is particularly important for starting formulas. These formulas have a casein/whey protein ratio of 40 : 60 or 50 : 50 and a total protein content of 1.3–1.6%. Follow-on formulas are higher in protein content (1.7–1.9%) and have sometimes the same casein/whey protein ratio as cow's milk.

77.4.1.1 Caseins. Caseins are the main protein fraction in bovine milk and can be separated from the whey proteins by acid precipitation at the isoelectric point (pH 4.6) or enzymatically by chymosine. All caseins are phosphorylated and especially the κ-casein is additionally glycosylated. Casein phosphopeptides which are released during the digestion can increase the calcium absorption which is important for growing infants. Furthermore, increased zinc absorption could be shown (Hansen and others 1996; Newburg 1997). The sugar structures of the κ-casein, especially the sialic acids seem to be effective in the prevention of the attachment of bacteria to the gastrointestinal mucosa (Lönnerdahl 2003).

TABLE 77.5 Human Milkborne Peptide Hormones and Hormonally Active Peptides (Peptides also Found in Bovine Milk Marked with #).

Hypothalamo-hypophysial hormones
Follicle stimulating hormone (FSH)
Gonadotropin releasing hormon (GnRH)
Growth hormone releasing factor (GRF)
Human growth hormone (GH)
Prolactin (PRL) #
HGH/PRLBP (binding peptide)
Thyrotropin releasing hormone (TRH) #
Thyroid stimulating hormone (TSH)
Calcitonin-like peptide
Parathyroid hormone-related peptide
Growth factors and cytokines
Acid growth factor (CAGF) = (EGF-like)
Basic growth factor (BAGF) = platelet differentiation GF-like)
Epidermal growth factor (EGF) #
Insulin
Insulin-like growth factor-I (IGF-I) #
Insulin-like growth factor-II (IGF-II) #
IGFBP-2, IGFB-3; not detected −1 and −4
Milk growth factor (MGF-S1, MGF-S2)
Milk derived growth factor 1
Neural growth factor (NGF)
Transforming growth factor (TGF α)
Transforming growth factor (TGF β) 1 and 2
Fibronectin stimulating activity (FnSA)
Granulocytes colony stimulating factor (GT-CSF)
Interleukins (IL 6, IL 8)
TNFα?
Gastrointestinal regulatory peptides
Gastrin
Gastric inhibitory peptide (GIP) #
Gastrin releasing peptide (GRP)
Motilin
Neurotensin
Peptide histidine methionine (PHM)
Peptide YY (PYY)
Somatostatin #
Vasoactive intestinal peptide (VIP)
Others
Atriopeptin (atrial factor; ANF)
Erythropoietin
Leptin
Melatonin
Relaxin #

77.4.1.2 Whey Proteins. For adaptation of the whey protein/casein ratio of infant milk formulas to human milk additional whey proteins are necessary. Such whey protein predominant formulas have had the disadvantage of causing hyperthreoninemia in infants (Moro and others 1989).

The threonine content in human milk proteins is similar to the threonine content of cow's milk proteins (Davis and others 1994). The reason for the hyperthreoninemias is

TABLE 77.6 Protein Composition of Human and Bovine Milk.

Proteins (g/100 mL)	Human Milk	Bovine Milk
Total protein	1.1	3.3
Casein	0.4	2.7
α-Casein	–	1.30
β-Casein	0.3	1.05
κ-Casein	0.1	0.35
Whey protein	0.7	0.6
α-Lactalbumin	0.3	0.13
β-Lactoglobulin	–	0.34
Serum albumin	0.05	0.04
IgA	0.1	0.003
IgG	0.001	0.08
Lactoferrin	0.2	0.01
Lysozyme	0.05	Trace

the glycomacropeptide (GMP) which is very rich in threonine and which increases the threonine content of whey predominant formulas for approximately 30% compared to human milk (Boehm and others 1998). GMP is released from casein during the enzymatic (chymosin) precipitation of κ-casein (Fig. 77.4). Because GMP does not precipitate it remains in the sweet whey fraction (Kawasaki and others 1994) (approximately 15 g/100 g whey protein) which is commonly used for the production of whey predominant infant milk formulas. Thus, the high threonine content of such formulas is a result of milk processing and not due to a high threonine content of bovine whey protein. The use of acid whey proteins which do not contain GMP can avoid the development of hyperthreoninemia in formula fed infants (Rigo and others 2001).

Infant formulas containing α-lactalbumin enriched whey proteins have the advantage of higher tryptophan contents similar to human milk. In the newborn, tryptophan and its metabolites like serotonin are essential for brain maturation and for the development of neurobehavioral regulations of food intake, satiation, and sleep–wake-rhythm. Today technological processes are available to adapt the amino acid composition of the formula according to the target composition.

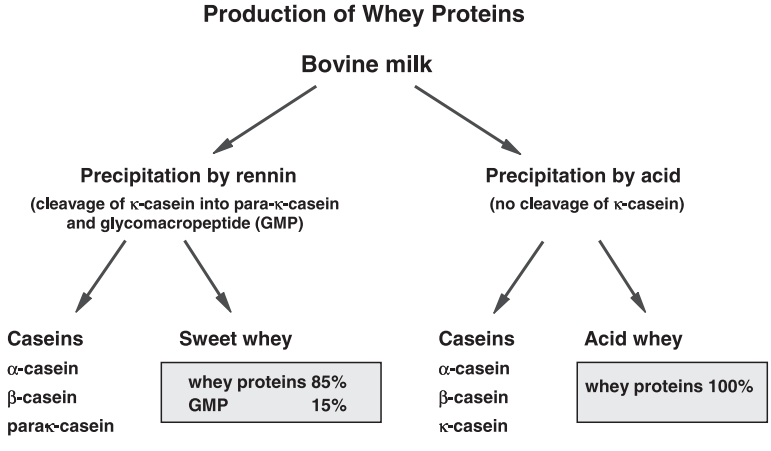

Figure 77.4 *Composition of sweet and acidic whey proteins with particular respect to the content of glycomacropeptide (GMP).*

77.4.1.3 Bovine Milk Protein Hydrolysates. For all infants with a cow's milk and/or soy protein allergy, products with hydrolyzed proteins are available. It can be distinguished between extensively hydrolyzed proteins or formulas based on free amino acids which are used for the treatment of allergies and partially hydrolyzed proteins used for allergy prevention in high risk infants (von Berg and others 2003).

The wide use of protein hydrolysate in infant formula led to the interesting question of the physiological role of peptides deriving during intestinal digestion of dietary proteins. In Tables 77.7 and 77.8 peptides are summarized which appear during digestion of either human milk proteins or bovine milk proteins. Although the tables are not complete they indicate very different functions which might be influenced by these peptides. In fact, in preterm infants an acceleration of the gastrointestinal transit of milk and stool and a more rapid establishment of full enteral feeding was observed with formulas based on hydrolyzed proteins (Mihatsch and others 2001, 2002) when compared to a formula based on the same protein source but without hydrolyzation. Such data indicate that these peptides can play a physiological role in the regulation of gastrointestinal functions as wells as its maturation.

77.4.2 Plant Proteins as Sources for Infant Formulas

77.4.2.1 Soy Proteins. Infant formulas based on soy proteins are widely used for infants suffering from cow's milk allergy particularly in the United States. However, up to 14% of the cow's milk allergic infants have also an allergy against soy proteins (Zeiger and others 1999). The biological quality of soy protein isolates ranges from 65% to 85% of that of casein (Muraro 2001). By addition of free amino acids like methionine, the quality can be made comparable to milk proteins.

Based on new technologies the phytate content of soy proteins could clearly be decreased resulting in a comparable, sometimes better absorption of calcium and zinc from infant soy formulas compared to cow's milk formulas (Boscher and others 1998).

Since several years isoflavones (phytooestrogens) in soy formulas are under discussion. The content of isoflavones is much higher in such formulas compared to cow's milk based formulas (Knight and others 1998). Not much is known about the oestrogenic effect of these phytooestrogens in infants. More clinical dates are necessary for a final evaluation of possible effects later in life caused by soy formulas.

77.4.2.2 Other Plant Proteins. More recently, other proteins deriving from plants are under discussion as protein sources for infant nutrition. Rice protein and potato protein are now adays technologically productible. However, relevant clinical data to evaluate the possible role of these new protein sources in infant nutrition are missing. Nevertheless, these new sources might be of interest in particular under the aspect of increasing allergy against nutritive proteins traditionally used in early life.

77.4.3 Analytical Techniques

77.4.3.1 Whey Proteins

α-Lactalbumin. α-Lactalbumin has been identified as a calcium metalloprotein, but can also bind other divalent cations (Hiraoka and others 1980). Several methods exist for the purification and quantification of α-lactalbumin. Chromatographic protocols use ion-pair

TABLE 77.7 Physiological Activities of Peptides Derived from Human Milk Digestion.

Bioacitve Peptide	Sequence	Effect
Opioid agonists		
β-Casomorphin-4	Tyr-Pro-Phe-Val [f51-4]	β-Casomorphins have only been found in the plasma of newborns. Reason may be the greater permeability of the newborn intestinal tract. Human β-casomorphins are less potent than bovine β-casomorphins
β-Casomorphin-4-amide (Valmuceptin)	[f51-4]	
β-Casomorphin-5	Tyr-Pro-Phe-Val-Glu [f51-5]	
β-Casomorphin-7	Tyr-Pro-Phe-Val-Glu-Pro-Ile [f51-7]	
β-Casomorphin-8	Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro [f51-8]	Antidiarrheal and sleep-inducing effects
β-Casomorphin-9	Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro-Tyr [f51-9]	
α-Lactorphin	Tyr-Gly-Leu-Phe [f50-3]	
β-Casomorphin	Tyr-Val-Pro-Phe-Pro [f158-62]	μ-Opioid receptor ligand with low potency
α_{S1}-Casomorphin amide	Tyr-Val-Pro-Phe-Pro-NH_2	Inhibition of the proliferation of T47D human breast cancer cells. No interaction with somatostatin receptors
		Inhibition of the proliferation of T47D human breast cancer cells. No interaction with somatostatin receptors
Opiod antagonists		
Casoxin C	Tyr-Pro-Tyr-Tyr [f31-4]	Opioid antagonist activity in the guinea pig ileum assay. Casoxin C has the same effect as naloxone
Lactoferroxin A	Tyr-Leu-Gly-Ser-Gly-Tyr	μ-Opioid receptor-selective antagonists with moderate potency
Lactoferroxin B	Arg-Tyr-Tyr-Gly-Tyr	μ-Opioid receptor-selective antagonists with moderate potency
Lactoferroxin C	Lys-Tyr-Leu-Gly-Pro-Gln-Tyr	μ-Opioid receptor-selective antagonists with moderate potency
Inhibition of ACE	Ser-Phe-Gln-Pro-Gln-Pro-Leu-Ile-Tyr-Pro [f43-52]	ACE inhibitory
Immunomodulatory effects		
β-Casein derived peptide	Val-Glu-Pro-Ile-Pro-Tyr [f54-9]	Stimulation of phagocytosis of sheep red blood cells by murine peritoneal macrophages
β-Casein derived peptide	Gly-Phe-Leu [f60-2]	Protective effect against bacterial (Klebsiella pneumoniae) infection in mice

(*Continued*)

TABLE 77.7 *Continued.*

Bioacitve Peptide	Sequence	Effect
α-Lactalbumin derived peptide	Gly-Leu-Phe [f51-3]	Protective effect against bacterial (Klebsiella pneumoniae) infection in mice
Calcitonin-gene-related peptide (CGRP)	Consist of 37 amino acids	Inhibitor of gastric acid secretion Involved in the protection of gastric mucosa from ulcerogenic agents. Present in human milk but not in infant formulas
Antimicrobial activity		
Lactoferricin	[f1-47]	Causes cell death by disruption of membrane integrity in Gram-negative bacteria
Antithrombotic activity		
Peptide derived from lactotransferrin	Lys-Arg-Asp-Ser	Inhibition of thrombin-induced serotonin release
κ-Caseinglycopeptide	[f39-42]	Inhibition of platelet-aggregation and fibrinogen-binding to ADP-activated platelets. Detected in plasma of newborns ingesting either formula or human milk
Sleep-inducing effects		
DSIP (delta-sleep-inducing peptide)		Circadian changes in concentration. High levels in colostrum. Part of the DSIP-LI in human milk seems to be present as free DSIP

TABLE 77.8 Physiological Activities of Peptides Derived from Bovine Milk Digestion.

Bioacitve Peptide	Sequence	Effect
Opioid agonists		
β-Casomorphin-4	Tyr-Pro-Phe-Pro [f60-3]	Most potent in inducing muscular rigidity
β-Casomorphin-4amide (Morphiceptin)	Tyr-Pro-Phe-Pro-NH_2 [f60-3]	Potent opioid activity both *in vivo* and *in vitro*. Most potent β-Casomorphin
β-Casomorphin-5	Tyr-Pro-Phe-Pro-Gly [f60-4]	Induces alterations of the amino acid transfer across the intestinal epithelium. Causes behavioral change (increases pain threshold) when injected systemically or ICV
β-Casomorphin-7	Tyr-Pro-Phe-Pro-Gly-Pro-Ile [f60-6]	Increases plasma prolactin release in rat
β-Casomorphin-11	[f60-70]	Proved to be an intestinal digestion product *in vivo*
α_{S1}-Casein-exorphin	Arg-Tyr-Leu-Gly-Tyr-Leu-Glu [f90-6]	Opioid agonist, efficient ligand for Cu^{II} ions
α-Lactorphin	Tyr-Gly-Leu-Phe [f50-3]	μ-Opioid receptor ligand with low potency
β-Lactorphin	Tyr-Leu-Leu-Phe [f102-5]	Induces contraction of guineapig ileum longitudinal muscle in the absence of electric stimulation and agonist. In addition antihypertensive activity
β-Lactotensin	His-Ile-Arg-Leu [f146-9]	Induces contraction of guineapig ileum longitudinal muscle in the absence of electric stimulation and agonist. Smooth muscle stimulating effect was not mediated by an opioid mechanism
κ-Casein glycomacropeptide	[f106-69]	Is supposed to induce the production of cholecystokinin
Opiod antagonists		
Casoxin-A	Tyr-Pro-Ser-Tyr-Gly-Leu-Asn [f35-41]	Opioid antagonist activity in the guinea pig ileum assay, Casoxin-A has the same effect as naloxone
Casoxin-B	Tyr-Pro-Tyr-Tyr [f58-61]	Opioid antagonist activity in the guinea pig ileum assay. Casoxin B has the same effect as naloxone
Casoxin-C	Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg [f25-34]	Agonist for C3a receptors. Ileum-contracting peptide. Phagocyte-stimulating activity
Inhibition of ACE		
α_{S1}-Casokinin-12	Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys [f23-34]	Casein dodeca peptide ("casein DP") launched in Japan, approved as a "Food for Specific Health Use"

(*Continued*)

TABLE 77.8 *Continued.*

Bioacitve Peptide	Sequence	Effect
α_{S1}-Casokinin-5	Phe-Phe-Val-Ala-Pro [f23-7]	ACE inhibitory. $IC_{50} = 6\ \mu M$
α_{S1}-Casokinin-6	Thr-Thr-Met-Pro-Leu-Trp [f194-9]	ACE inhibitory. $IC_{50} = 16\ \mu M$
Peptides derived from β-casein	Lys-Val-Leu-Pro-Val-Pro-Gln [f169-75] Lys-Val-Leu-Pro-Val-Pro [f169-74]	Significant antihypertensive effect in SHR. Pancreatic digestion leads to a hexapeptide with stronger antihypertensive activity ($IC_{50} = 5\ \mu M$)
Peptides derived from β-casein	Val-Tyr-Pro-Phe-Pro-Gly [f59-64] Val-Tyr-Pro [f59-61] Thr-Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro [f80-90] Phe-Pro [f62-3;157-8;205-6]	ACE inhibitory
β-Lactoglobulin derived peptide	Ile-Pro-Ala [f78-80]	ACE inhibitory
β-Lactoglobulin peptide	Ala-Leu-Pro-Met-His-Ile-Arg [f142-8]	ACE-inhibition index of 84.3%. Resistant to further digestion with pepsin
α-Lactalbumin derived peptides	Tyr-Gly Leu-Phe	ACE inhibitory
Albutensin A	Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg [f208-216]	ACE inhibitory, ileum contracting and relaxing activcities
Peptides derived from α_{S1}- and β-caseins	Pro-Pro-Gln-Ser-Val-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Val-Pro-Glu Leu-Leu-Tyr-Gln-Gln-Pro-Val-Leu-Gly-Pro-Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val Asp-Glu-Leu-Gln-Asp-Lys-Ile-His-Pro-Phe-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser	Effective ACE inhibitory activity in SHR rats
Calpis™ sour milk	Val-Pro-Pro Ile-Pro-Pro	Inhibition of ACE in the abdominal aorta of spontaneously hypertensive rats. Blood pressure of hypertensive human subjects was significantly reduced following daily ingestion of 95 mL sour milk for 2 months
Immunostimulatory effects		
α_{S1}-Casein derived peptide	Thr-Thr-Met-Pro-Leu-Trp [f194-9]	Stimulation of phagocytosis of sheep red blood cells by murine peritoneal macrophages. Protective effect against bacterial (*Klebsiella pneumoniae*) infection in mice

(*Continued*)

TABLE 77.8 *Continued.*

Bioacitve Peptide	Sequence	Effect
Isradicin	[f1-23]	Protected mice against *Staphylococcus aureus* and *Candida albicans* by stimulation of both phagocytosis and immune responses
β-Casein derived peptides	Pro-Gly-Pro-Ile-Pro-Asn [f63-8] Leu-Leu-Tyr [f191-3]	Stimulation of phagocytosis of sheep red blood cells by murine peritoneal macrophages. Protective effect against bacterial (*Klebsiella pneumoniae*) infection in mice
κ-Casein and α-lactalbumin derived peptides	Tyr-Gly [f38-9] Tyr-Gly [f18-9; f50-1] Tyr-Gly-Gly [f18-20]	Enhance the proliferation of human peripheral blood lymphocytes
Lactoferrin pepsin hydrolysate (LFH)		Enhancement of mucosal immunity
Tuftsin	Thr-Lys-Pro-Arg	Enhancement of neutrophil and macrophage chemotoxis, cytotoxic activity of macrophages and natural killer cells, antigen processing
Immunosuppressive effects		
Glycomacropeptide (CMP)	[f106-69]	*In vitro* inhibition of the mitogen-induced proliferation of mouse spleen B lymphocytes and rabbit Payer's patch cells. Suppression of IgG class antibody production
Antimicrobial activity		
Lactoferricin B	[f17-41] [f17-31]	Bacterial activity against gram negative bacteria (e.g., *E. coli, Kl. pneumonia, Sal. enteritidis, St. haemo-lyticus, Str. thermophilus, Corynebacterium ammoniagenes, B. subtilis, Bifidobacterium infantis*), some yeasts, Gram-positive bacteria, fungi, protozoa and tumors. No effect against *Bifidobacterium*
Casocidin-I	f165-203	Inhibits the growth of *E. coli* and *Staphylococcus carnosus*
κ-Casein glycomacropeptide	[f106-69]	Exerts antiviral effects by inhibiting haemagglutination of influenza virus or by blocking receptor to cholera toxin
Probiotic effects		
κ-Casein glycomacropeptide	[f106-69]	Enhances growth of Bifidobacteria, especially *Bifidobacterium infantis* S_{12}

(*Continued*)

TABLE 77.8 *Continued.*

Bioacitve Peptide	Sequence	Effect
Antithrombotic activity		
Casoplatelins	Undecapeptide: Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys [f106-16] Pentapeptide: Lys-Asn-Gln-Asp-Lys [f112-6] Caseinglycopeptide: [f106-69]	Inhibitors of the aggregation of ADP-activated platelets as well as binding of human fibrinogen γ-chain to a specific receptor site on the platelet surface
Mineral binding porperties		
α_{S1}-Casein-exorphins	Arg-Tyr-Leu-Gly-Tyr-Leu-[Glu] [f90-5; 90-6]	Efficiant ligands for Cu^{II} ions
α_{S1}-Casein phosphopeptides	[f43-58] [f59-79] Ser-Ser-Ser-Glu-Glu-Ile-Val-Pro-Asn [f66-74]	Calcium-binding phosphopeptides can have an anticariogenic effect through recalcification of the dental enamel
α_{S2}-Casein peptides	[f46-70] [f1-21] [f2-21]	Formation of organophosphate salts, may function as carrier for different minerals, especially calcium
β-Casein phosphopeptides	[f1-25] [f1-28]; [f33-48]	Formation of organophosphate salts, may function as carrier for different minerals, especially calcium
Calmodulin-binding properties		
	Leu-Lys-Lys-Ile-Ser-Gln-Arg-Tyr-Gln-Lys-Phe-Ala-Leu-Pro-Gln-Tyr [f164-79] Val-Tyr-Gln-His-Gln-Lys-Ala-Met-Lys-Pro-Trp-Ile-Gln-Pro-Lys-Thr-Lys-Val-Ile-Pro-Tyr-Val-Arg-Tyr-[Leu] [f183-207] [f183-206]	Inhibition of calmodulin-dependent cyclic nucleotide phosphodiesterase activity

reversed phase HPLC (Neyestani and others 2003) or immobilized metal ion affinity chromatography (IMAC) (Blomkalns and Gomez 1997). α-Lactalbumin may also be quantified by an enzyme-linked immunosorbent assay (ELISA) technique.

β-Lactoglobulin. Quantification of β-lactoglobulin is conveniently carried out by ELISA technique for which commercially available test kits exist. Qualitative analysis of β-lactoglobulin during purification and isolation can be accomplished by gel electrophoresis but also liquid chromatographic techniques like ion exchange or reversed phase chromatography.

Lactoferrin. Various analytical techniques for the determination of lactoferrin are reported. High performance liquid chromatography (HPLC) or fast protein liquid

chromatography (FPLC) in combination with ion-exchange chromatography is applied to isolate lactoferrin (Eigel and others 1984; Ekstrand and Björck 1986), due to the high isoelectric (Ekstrand and Björck 1986). It can also be measured by ELISA.

77.4.3.2 Caseins. The casein components exist in strong association with each other as a micellar complex stabilized by van der Waals forces, hydrophobic interactions, hydrogen bonding, electrostatic, and steric stabilization. For the purification and isolation of individual caseins it is necessary to eliminate these protein–protein interactions. Various concentrations of urea, dimethylformamide, β-mercaptoethanol, or dithiothreitol are used in protocols to primarily cleave hydrogen bonds and reduce disulfide linkages (Bramanti and others 2002). Separation and determination of bovine caseins can be achieved by hydrophobic interaction chromatography. Alternatively the caseins can be isolated by FPLC (Hollar and others 1991) using a strong cation exchanger.

The glycomacropeptide (GMP) can be prepared by cation exchange processes taking advantage of the highly negative charge on GMP even at low pH (Kawasaki and others 1994) where the other whey proteins are positively charged. Alternatively, whey at pH less than 4 is contacted with an anion exchanger. GMP can also be purified by IMAC on copper containing affinity supports (Etzel 1999) or solely by an ultrafiltration process of sweet whey at a pH below 4 (Tanimoto and others 1991).

77.4.3.3 Milk Protein Hydrolysates. For the characterization of protein hydolysates the degree of hydrolysis (DH) is most often applied. The DH is defined as percentage of peptide bonds cleaved, thus $DH = (h/h_{total}) \times 100\%$, where h is hydrolysis equivalent defined as concentration in milliequivalents per g of protein of α-amino groups formed during hydrolysis and h_{total} is hydrolysis equivalent at complete hydrolysis to amino acids. h_{total} is calculated by summing up the contents of individual amino acids per g of protein. Traditionally, DH can be measured with the trinitro benzene sulfonic acid method as described by Adler-Nissen (1979). Along with the DH the molecular weight distribution of the hydrolysate is used for characterization which can be done by gel filtration. To describe a protein hydrolysate it is also important to know the amount of free amino acids. Free amino acids can be determined by reversed-phase HPLC with precolumn derivatization or ion exchange chromatography.

77.4.4 Future Trends

Based on the long experience with measurements of protein metabolism the nutritive quality of the dietary proteins is nearly simmilar than that of human milk proteins. However, there is an intensive discussion ongoing concerning the relationship of early growth veloicity and the incidence of metabolic disorders like obesity in later life (Metges 2001; Hoppe and others 2004). Because growth velocity depends mainly on protein intake the target protein concentration for infant formulas are under reevaluation. Although this discussion is still on a very early stage and the mechanisms behind are not identified first products are on the market that claim the beneficial effect of low protein concentration in infant formula.

Due to the increasing incidence of allergic diseases in the developed countries the hypoallergenic formulas play an important role for prevention of allergic diseases. Although products with this type of protein are since more than 25 years on the market only few valid data exists about their preventive effects. There is consensus that in

cases of infants with a risk for bovine milk allergy a hypoallergenic product should be used. However, in infants at risk for other forms of allergy or atopy the preventive effect is still under debate.

77.5 NEXT STEPS FOR DEVELOPMENT OF FUNCTIONAL COMPOUNDS FOR INFANT FORMULAS

The fact that diseases later in life can be influenced by nutrition during infancy has raised a completely new perspective regarding the judgement of infant formulas. Basically, the discussion is not new: Effects of LCPUFA on brain development or the consequences of pre- or probiotics on the development of the immune system are current examples, which have been extensively reviewed in this chapter. However, starting point of the development were the current requirements of the infant rather than the prevention of later diseases. Therefore, the composition of human milk served as an example. This development has not been finalized, yet.

Thus, the question still remains why there is such a huge structural variety of the oligosaccharide fraction of human milk. It may be assumed that a great variety of functions is associated with the large number of structures and largely exceeds the prebiotic concept. For instance, in experimental trials direct effects of oligosaccharides on the cells of the immune system have been found. Should this be confirmed, the existing concept of allergy prevention by avoidance and reduction of antigen load would have to be reassessed.

In future, the concept of prevention will play an ever increasing role. As a result, diseases that are important in adulthood will be the focus of attention. It has to be assumed that the prevention of civilization diseases was of minor importance during the evolutionary development of human milk. Thus, it could happen that on developing infant nutrition for the prevention of civilization diseases, the composition of human milk would no longer serve as an example.

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78

Biochemical Processes in the Production of Flavor in Milk and Milk Products

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78.1 Introduction 716
78.2 Reactions Involved in the Production of Flavor Compounds in Milk and Milk Products 716
78.2.1 Thermally-Induced Changes 717
78.2.2 Lipid Oxidation 717
78.2.3 Fermentation by Lactic Acid Bacteria 718
78.2.3.1 Glycolysis and Related Reactions 719
78.2.3.2 Citrate Metabolism 719
78.2.3.3 Lipolysis and Related Reactions 719
78.2.3.4 Proteolysis and Related Reactions 722
78.3 Characterization of Flavor of Milk and Milk Products 726
78.3.1 Chemical Anchors for Specific Flavors or Off-Flavors in Milk and Milk Products 733
78.3.2 Taste Compounds in Milk and Milk Products 736
78.4 Conclusions 739
References 740

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78.1 INTRODUCTION

Fresh milk (raw or pasteurized) of overall good flavor quality has a bland but characteristic flavor (Badings 1991). Various dairy products made from this basic raw material can be described as mild/weak (e.g., yogurt, mozzarella cheese) to intensely flavored (e.g., concentrated/evaporated milk, blue cheeses, Italian hard cheeses). The overall sensory experience is composed of three elements: (1) the mouthfeel from the constituents of the milk, especially milk fat and proteins which are essential for the viscosity and/or texture, (2) taste components, for example, a slight sweet/salty taste that is due to lactose, milk salts, and added salt (NaCl), and (3) aroma caused by proper balance of numerous volatile organic compounds.

In the past, the stability of milk and milk products was the primary consideration, but is no longer the principal objective due to the evolution of modern sanitary practices, as well as pasteurization. Today, the manufacture of dairy products of consistently good flavor and texture is crucial. In previous flavor research, researchers had identified hundreds of volatile compounds, with little or no attention paid to their sensory contribution to the overall flavor of the products. The availability of powerful chromatographic separation techniques like capillary gas chromatography in combination with mass spectrometry and olfactory detection techniques (sniffer ports), has revolutionized the work on characterization of flavor compounds. Advancements in instrumental/chemical analysis have paralleled the developments in sensory methods for the analysis of flavor compounds. Recently, published reviews by Parliment and McGorrin (2000), McGorrin (2001), and Singh and others (2003a) described various sensory-directed analytical flavor techniques used in the evaluation of key aroma compounds in milk and dairy products.

This chapter presents a discussion on the aroma/taste compounds of various dairy products and reactions involved with their production. Specific flavor notes, off-flavors, and their causative agents are also discussed.

78.2 REACTIONS INVOLVED IN THE PRODUCTION OF FLAVOR COMPOUNDS IN MILK AND MILK PRODUCTS

The volatile flavor and taste compounds in milk products originate from degradation of the major milk constituents, namely lactose, citrate, milk lipids, and milk proteins (collectively called caseins). The physicochemical parameters, that is, heat treatment, pH, water activity, salt concentration, and ripening temperature, necessary for the right balance of biochemical changes are set during manufacturing. In case of deviation of any of these parameters, cheeses or other dairy products could potentially develop texture and/or flavor inconsistencies. Degradation of milk constituents during manufacture of dairy products involves a concerted series of chemical and biochemical reactions. Reactions and/or pathways involved in the production of milk and milk products can be subdivided into three major categories:

1. Thermally-induced changes
2. Lipid oxidation
3. Fermentation by lactic acid bacteria.

78.2.1 Thermally-Induced Changes

Thermal treatment of fluid milk, such as pasteurization or production of various concentrated/dried milk products, leads to a whole series of desirable and undesirable chemical changes which have major consequences for the texture, taste, and flavor. Selected changes, which directly influence the flavor/off-flavor of milk and milk products, are outlined below:

- Thermal degradation of lipids (e.g., accelerated degradation of hydroperoxides resulting in formation of 2-alkanones). For details on thermal degradation of lipid see Nawar (1989) and Yoo and others (1989).
- Thermal reactions involving amino side chains (e.g., generation of H_2S and other S-compounds).
- Maillard reactions: reaction of lactose, a reducing sugar, with most ε-amino groups on the side chain of lysine residues in milk proteins (e.g., resulting in the formation of a wide variety of odorants, namely furfurals, 3-/4-furanones, Strecker aldehydes, pyrazines, pyrroles, thiazoles, maltol, furaneol, etc.) (see Fig. 78.1).

This is in no way the complete list of thermally induced reactions in milk and milk products. Many other reactions, such as hydrolysis of peptide bonds, dephosphorylation of proteins, and so on, occur but are not important from a flavor point of view.

78.2.2 Lipid Oxidation

Autoxidation of milk fat in milk and milk products usually starts with the phospholipids of the milk fat globule membrane. These lipids have highly unsaturated fatty acid residues.

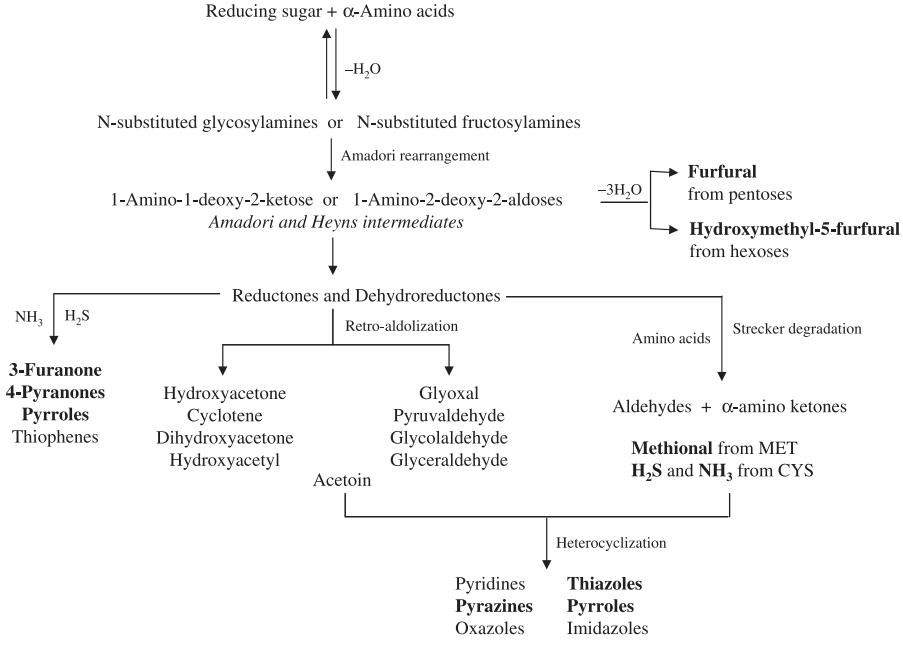

Figure 78.1 *Formation of aroma compounds via Maillard reaction/non-enzymatic browning.*

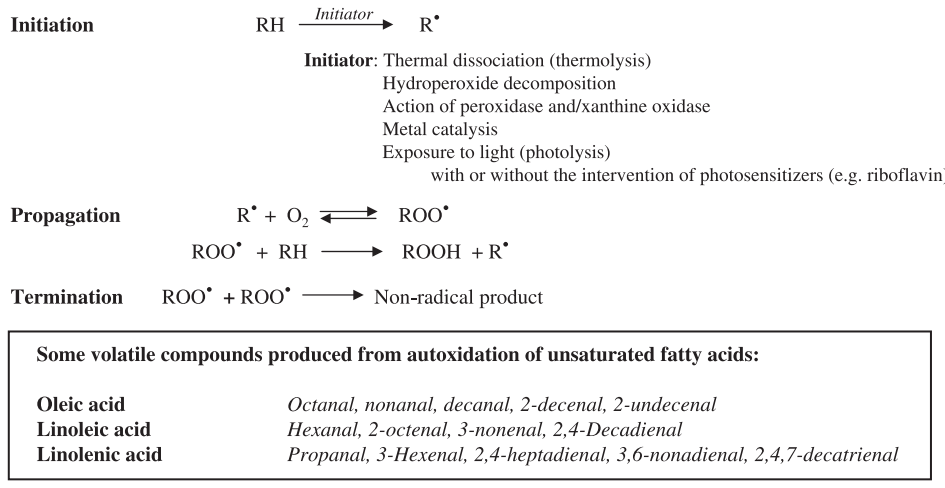

Figure 78.2 *General pathway way for the autoxidation of unsaturated lipid, with some examples of volatiles produced from individual unsaturated fatty acid. Information compiled from Frankel (1980), Ho and Chen (1994), and Lee and Morr (1994).*

A simplified scheme of lipid oxidation involving three distinct steps is put together with the oxidation products of fatty acids (see Fig. 78.2) (for further details see Frankel 1980; Walstra and others 1999). Some of these oxidation products can be perceived in exceptionally low concentrations and thereby cause off-flavors, described as fatty, fried, plastic, tallowy, fishy, metallic, and cardboard-like. Off-flavor development can cause problems in fluid milk, sour cream buttermilk, cream, butter, butter oil, milk powders, cheeses, and casein and whey protein products.

78.2.3 Fermentation by Lactic Acid Bacteria

Considerable knowledge on the principal changes and pathways involved in manufacture of fermented milk and cheese ripening has been accumulated over the last several decades. The three primary biochemical processes are:

- Glycolysis
- Lipolysis
- Proteolysis.

Their relative importance depends on the type of fermented dairy product. These primary changes are followed and overlapped by a host of secondary catabolic changes, including deamination, decarboxylation, and desulfurylation of amino acids; β-oxidation of fatty acids; and even some synthetic changes, for example, esterification. Most of these changes involve biochemical reactions, but some may be purely chemical. The abovementioned primary reactions are mainly responsible for the basic textural changes and are also largely responsible for the basic flavor of fermented dairy products. However, the secondary transformations are mainly responsible for the finer aspects of cheese flavor and modify cheese texture. In the next few sections, glycolysis, lipolysis, proteolysis, and other related reactions are discussed.

78.2.3.1 Glycolysis and Related Reactions. During fermented milk and cheese manufacture, starter lactic acid bacteria ferment lactose to (mainly L) lactic acid (Fig. 78.3). In the case of Cheddar-type cheeses, most of the lactic acid is produced in the vat before salting and molding. During manufacture or shortly thereafter, curd pH reaches ~5.0, but the rate is characteristic of cheese variety (6–24 h). Even after losing ~98% of the total milk lactose in the whey as lactose or lactate, the cheese curd still contains 0.8–1.5% lactose at the end of manufacture (Huffman and Kristoffersen 1984).

The pH at whey drainage largely determines the mineral content of a cheese. The loss of Ca^{2+} and phosphate from casein micelles determines the extent to which the micelles are disrupted and this largely determines the basic structure and texture of a cheese (Lawrence and others 1983). In general, curds with a low pH, for example, Cheshire, have a crumbly texture, while high pH curds, for example, Emmental, tend to be more elastic.

The racemization of L-lactate is probably not significant from a flavor viewpoint, but D-lactate may have undesirable nutritional consequences in infants. Calcium D-lactate is believed to be less soluble than calcium L-lactate and may crystallize in cheese, especially on cut surfaces (Dybing and others 1988). Consumers may mistake the crystals as spoilage, and crystal formation is generally considered negative.

Lactic acid is further metabolized by propionic acid bacteria, for example, in the production of Swiss-type cheeses, to propanoic acid and carbon dioxide (CO_2). The production of CO_2 is responsible for the eye formation which is a characteristic of Swiss-type cheeses (Fox and others 1995). Oxidation of lactate can also occur in cheese. During this process, lactate is converted to acetate and CO_2. Acetate is present at fairly high concentrations in Cheddar and is considered to contribute to cheese flavor, although high concentrations of acetate may cause off-flavors (Aston and Dulley 1982).

78.2.3.2 Citrate Metabolism. Bovine milk contains relatively low levels of citrate (~8 mM). Approximately 90% of the citrate in milk is soluble and most is lost in the whey; however, the concentration of citrate in the aqueous phase of cheese is ~3 times that in whey (Fryer and others 1970), presumably reflecting the concentration of colloidal citrate. Cheddar cheese contains 0.2–0.5% (w/w) citrate, which is not metabolized by *Lc. lactis* ssp. *lactis* or ssp. *cremoris*, but is metabolized by *Lc. lactis* biovar *diacetylactis* and *Leuconostoc* ssp., with the production of diacetyl and CO_2. Due to CO_2 production, citrate metabolism is responsible for the characteristic eyes in Dutch-type cheeses. Diacetyl and acetate produced from citrate contribute to the flavor of Dutch-type and cheddar cheeses (Aston and Dulley 1982). Several species of mesophilic lactobacilli metabolize citrate with the production of diacetyl and formate (Fryer 1970); the presence of lactose influences the amount of formate formed.

The principal flavor compounds produced from metabolism of citrate are acetate, diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), and 2,3-butandiol (Cogan 1995) (see Fig. 78.4.) Diacetyl is usually produced in small amounts but acetoin is generally produced in much higher concentration (10–50-fold higher than diacetyl). Acetate is produced from citrate in equimolar concentrations.

78.2.3.3 Lipolysis and Related Reactions. Like all types of food with a high fat content, lipolytic (enzymatic hydrolysis by lipases and esterases) and oxidative (chemical) changes are likely to occur in dairy products. Lipases and esterases in cheese originate from milk, starter, and secondary starter and nonstarter bacteria. A number of

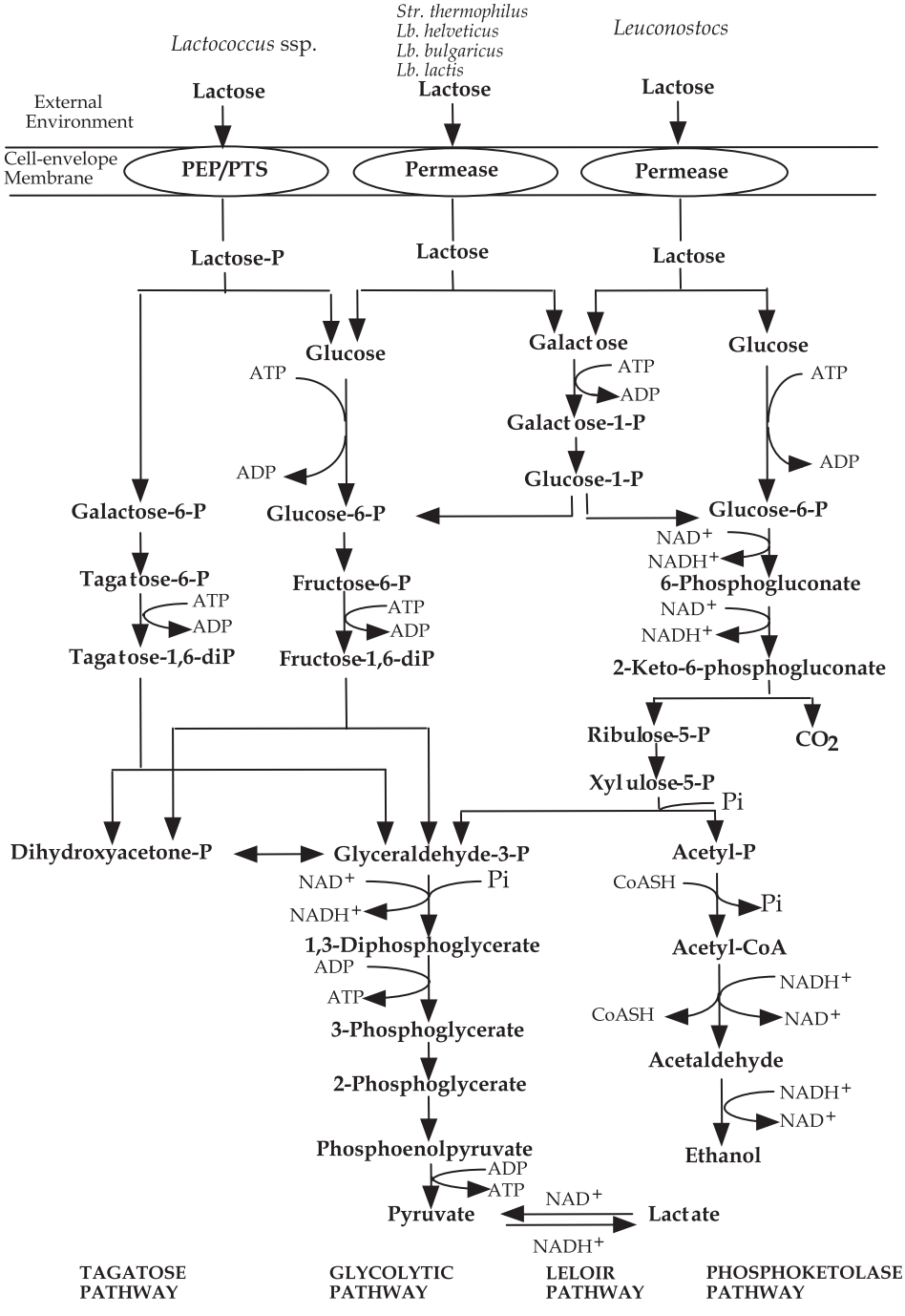

Figure 78.3 *Probable pathways for the metabolism of lactose by mesophilic and thermophilic lactic acid bacteria. (Adapted from Fox and others 1990.)*

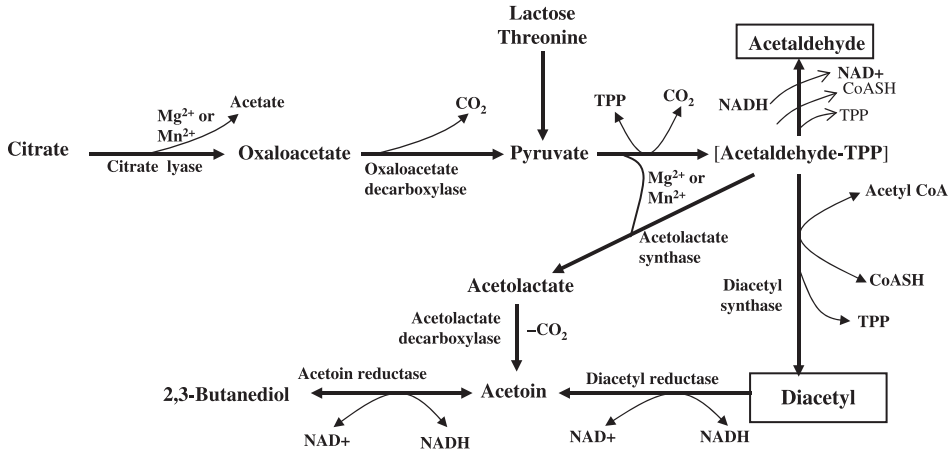

Figure 78.4 *Metabolism of citrate by Lactic acid bacteria. (Adapted from Fox and others 1995; Walstra and others 1999.)*

psychrotrophic organisms, which can dominate the microflora of refrigerated milk, produce heat-stable lipases. The hydrolysis of triglycerides, which constitute more than 98% of milk fat, is the principal biochemical transformation of fat, which leads to the production of free fatty acids (FFA), di- and monoglycerides and possibly glycerol (Fig. 78.5). FFA contribute to the aroma of cheese. Individual FFA, particularly acids between $C_{4:0}$ and $C_{12:0}$, have specific flavors (rancid, sharp, goaty, soapy, coconut-like). The flavor intensity of FFA depends not only on the concentration but also on the distribution between aqueous and fat phases, the pH of the medium, the presence of certain

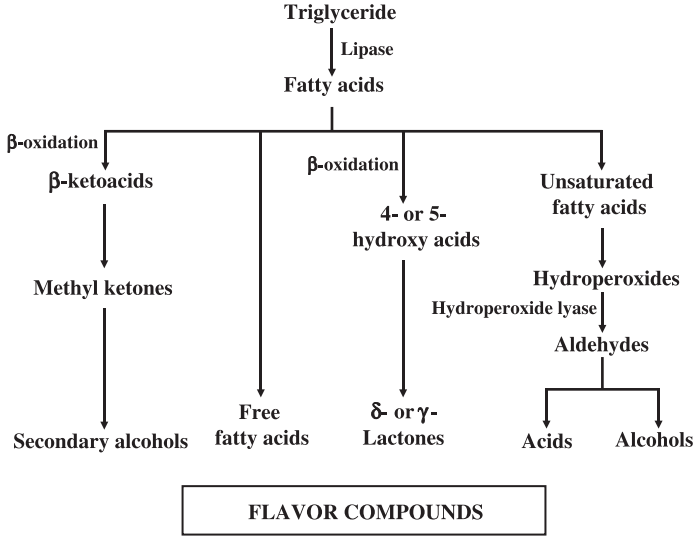

Figure 78.5 *General pathways for the metabolism of milk triglycerides and fatty acids.*

cations (e.g., Na^+, Ca^{2+}), and protein degradation products (Adda and others 1982). The pH has a major influence on the flavor impact of FFA. At the pH of Cheddar (pH ~5.2), a considerable portion of FFA are present as salts, which are nonvolatile, thus reducing their flavor impact. In most cheese varieties, relatively little lipolysis occurs during ripening and too much is considered undesirable. Most consumers would consider Cheddar, Dutch, and Swiss-type cheeses containing even moderate levels of free fatty acids to be rancid. Even lesser amounts of FFA would make fermented milk, such as yogurt, rancid. However, extensive lipolysis is desirable as part of overall flavor development in certain cheeses, such as hard Italian cheeses (Romano, Provolone), blue, and feta.

The fat fraction of the dairy product is also important for the development of typical flavor. Cheddar cheese made from nonfat milk does not develop full aroma, even after 12 months (Ohern and Tuckey 1969). Foda and others (1974) also suggested that the fatty acid composition and natural emulsion of milk fat are important for flavor development.

Metabolism of Fatty Acids. FFA are involved in several types of reactions which vary in importance with the type of dairy product involved (Fig. 78.5). Methyl ketones are produced from fatty acids by oxidative degradation. The production of methyl ketones involves oxidation of fatty acids to β-ketoacids, which are then decarboxylated to corresponding methyl ketones with one carbon atom less, mainly from $C_{6:0}$ to $C_{12:0}$ fatty acids (Hawke 1966). Methyl ketones are responsible for the characteristic aroma of blue-veined cheeses (Gripon and others 1991). However, they do play a limited role in Cheddar cheese flavor. Ultimately, methyl ketones can be reduced to secondary alcohols, which do not contribute to cheese aroma. Another reaction in which polyunsaturated and, perhaps, monounsaturated fatty acids can be involved is oxidation. The extent of oxidation in cheese is, however, rather limited, possibly due to a low redox potential. This, together with the presence of natural antioxidants, could prevent the initiation of oxidation mechanisms, or create conditions in which the primary oxidation products are reduced (Adda and others 1982).

Aliphatic and aromatic esters play an important part in the flavor and, sometimes, the off-flavor of cheese. This synthesis mainly concerns the above mentioned short- or medium-chain fatty acids and the alcohols involved may be aliphatic (ethanol), aromatic (phenylethanol), or thiols (methanethiol). Esters can be produced enzymatically, by lactic acid bacteria (Hosono and others 1974; Harper and others 1980) but can also easily result from a purely chemical reaction. Esters generally contribute a fruity flavor to dairy products which is desirable and characteristic in many cheeses (Parmesan, Parrano) but undesirable in others (cheddar).

γ- and δ-lactones have been identified in cheeses, particularly in cheddar, where they have been considered as important for flavor (Wong and others 1973; Drake and others 2001). Lactones are cyclic esters resulting from the intramolecular esterification of hydroxy acids through the loss of water to form a ring structure. Lactones possess a strong aroma, which although not specifically cheese-like, may be important in the overall cheese flavor impact.

78.2.3.4 Proteolysis and Related Reactions. Proteolysis is the most widely studied biochemical change. During the manufacture of fermented milks and cheeses and during ripening of cheeses, a gradual decomposition of caseins occurs due to the

combined action of various proteolytic enzymes. These generally include enzymes from the following sources:

- Coagulant
  a. Chymosin (genetically engineered)
  b. Chymosin/pepsin (from calf stomach)
- Indigenous milk enzymes
  a. Plasmin
  b. Cathepsin
- Starter and nonstarter bacterial enzymes
  a. Cell envelope-associated proteinases (CEP)
  b. Peptidases
     i. Endopeptidases
     ii. Aminopeptidases
     iii. Di- and tri-peptidases
     iv. Proline specific peptidases
- Secondary starter enzymes.

Enzymes from the first three sources are active in most ripened cheeses. The secondary starter (microorganisms added to cheese milk or curd for purposes other than acidification, for example, surface smear organisms, blue/white molds) exerts considerable influence on the maturation of cheese varieties in which they are used. Exogenous enzymes used to accelerate ripening could be added to the above list, and when present can be very influential.

The correct pattern of proteolysis is generally considered to be a prerequisite for the development of the correct flavor of cheese. Products of proteolysis per se (i.e., peptides and free amino acids) probably are significant in cheese taste, at least to "background" flavor, and some off-flavors, for example, bitterness, but are unlikely to contribute aroma. Compounds arising from the catabolism of free amino acids contribute directly to cheese taste and aroma.

In the cheese environment, with a high ionic strength and a low a_w, rennet-induced breakdown of α_{s1}-casein proceeds much faster than that of β-casein (α_{s2}-and κ-caseins are quite resistant to hydrolysis by the rennet) (Visser 1993). The residual chymosin rapidly hydrolyses α_{s1}-casein at the bond Phe^{23}–Phe^{24} during the initial stages of ripening (Creamer and Richardson 1974). Hydrolysis of this single bond of α_{s1}-casein causes a rapid change in the rubbery texture of young Cheddar curd into a smoother, more homogeneous product (Lawrence and others 1987). The peptide α_{s1}-CN f1-23, produced by chymosin action on the bond Phe^{23}–Phe^{24} of α_{s1}-casein, is further hydrolyzed in Cheddar cheese (Singh and others 1994) by CEP from starter *L. lactis* ssp. *cremoris* resulting in the production of whole range of small molecular weight peptides. The small peptides from α_{s1}-CN f1-23 representing N-terminal (α_{s1}-CN f1-7, 1-9, 1-13, and 1-14) and C-terminal (α_{s1}-CN f14-17, 17-21) sequences were found to be bitter in taste (Lee and others 1996; Richardson and Creamer 1973). Chymosin produced large peptide α_{s1}-CN f24-199 is further hydrolyzed by chymosin and CEP (for further details see McSweeney and others 1994; Singh and others 1995, 1997).

Chymosin has limited action on β-casein in Cheddar, although some activity is indicated by the presence of the peptide β-CN f1-192 (McSweeney and others 1994). Hydrolysis of the bond Leu^{192}–Tyr^{193} of β-casein by chymosin releases a small corresponding C-terminal fragment, β-CN f193-209, which is extremely bitter (Singh and others 2004b). Nearly half of the β-casein in Cheddar cheese is hydrolyzed during the ripening. Plasmin, an indigenous milk proteinase, is mainly responsible for the initial proteolysis of this protein. Plasmin hydrolysis of β-casein results in the formation of three γ-caseins [γ_1- (β-CN f29-209), γ_2- (β-CN f106-209), and γ_3- (β-CN f108-209) caseins], representing C-terminal region, and five proteose-peptones [β-CN f1-28, β-CN 1-105/107, and β-CN f29-105/107] representing the corresponding N-terminal region. The γ-caseins seem to accumulate in Cheddar over the ripening period. The proteose-peptones are extensively hydrolyzed by the starter bacterial CEP and peptidases to produce small peptides and free amino acids (Singh and others 1995, 1997).

Proteolysis in cheese seems to be a sequential process involving rennet, milk proteinase (particularly plasmin), the starter culture, secondary microorganisms, and NSLAB. The hydrolysis of casein to high molecular weight peptides is thought to be primarily the result of chymosin and plasmin (Singh and others 2003a). The subsequent hydrolysis of high molecular weight peptides is primarily the result of proteolytic enzymes from lactic acid bacteria. Proteolytic degradation of caseins into peptides in Cheddar (Singh and others 1994, 1995, 1997; Fernandez and others 1998), Emmentaler (Gagnaire and others 2001), and Parmigiano-Reggiano cheeses (Addeo and others 1992, 1994) were characterized in detail.

Metabolism of Amino Acids. In lactococci, the first step in the degradation of amino acids is transamination (see Fig. 78.6; Gao and others 1997), leading to formation of α-keto acids (α-KA). Aromatic aminotransferase enzymes have been previously characterized from *Lactococcus lactis* ssp. *cremoris* (Yvon and others 1997; Rijnen and others 1999a) and *Lactococcus lactis* ssp. *lactis* (Gao and Steele 1998). These enzymes initiate the degradation of Val, Leu, Ile, Phe, Tyr, Trp, and Met, all of which are known precursors of cheese flavor compounds. Inactivation of aminotransferase enzymes involved in the breakdown of amino acids by lactococci has been shown to reduce aroma formation during cheese ripening (Rijnen and others 1999b).

The volatile fraction of cheese has several sulfur-containing compounds, such as methanethiol, methional, dimethyl sulfide, dimethyldisulfide, dimethyltrisulfide, dimethyltetrasulfide, carbonyl sulfide, and hydrogen sulfide (Lindsay and Rippe 1986; Urbach 1995; Weimer and others 1999) and they contribute to the aroma of cheese (Milo and Reineccius 1997). These compounds are known to originate from Met. Methanethiol has been associated with desirable Cheddar-type sulfur notes in good quality Cheddar cheese (Manning and Price 1977; Manning and Moore 1979; Price and Manning 1983). However, alone or in excess, methanethiol does not produce typical Cheddar cheese flavor (Weimer and others 1999).

Methanethiol is readily oxidized to dimethyl disulfide and dimethyl trisulfide (Parliment and others 1982; Chin and Lindsay 1994). Occurrence of these compounds is a direct result of methanethiol content and is modulated by the low redox potential present in cheese. Methanethiol can potentially oxidize during analysis to form these compounds, and this may account for some reports of dimethyl disulfide and dimethyl trisulfide in cheese. Dimethyl sulfide (Milo and Reineccius 1997) and dimethyl trisulfide were

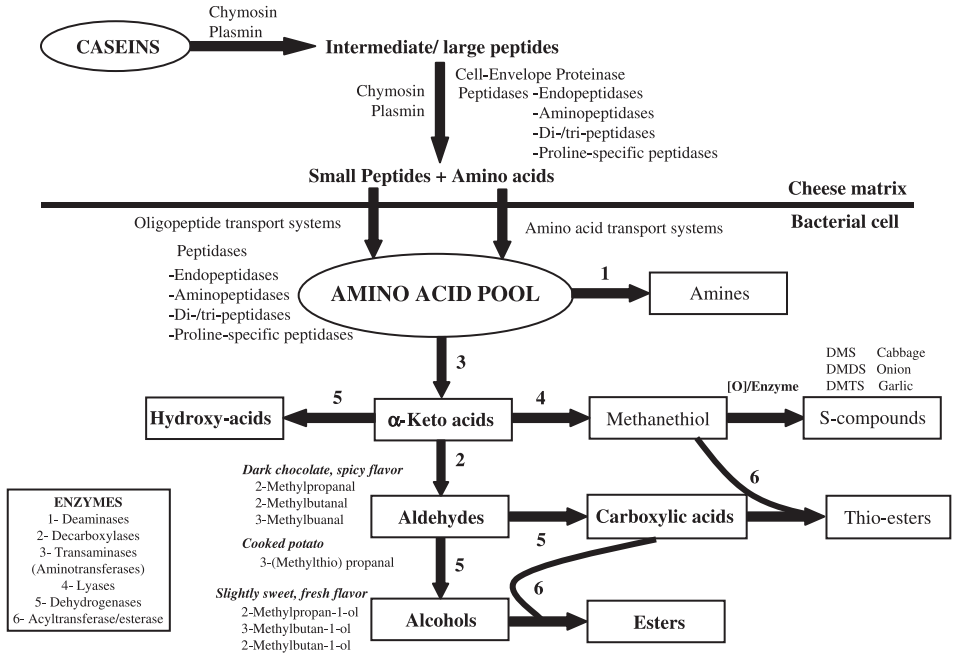

Figure 78.6 *Generation of flavor compounds from milk protein degradation. DMS, dimethyl sulfide; DMDS, dimethyl disulfide; DMTS dimethyl trisulfide. (Modified from Kranenburg and others 2002.)*

recently noted as important odorants in aged Cheddar cheese (Milo and Reineccius 1997; Suriyaphan and others 2001b; Zehentbauer and Reineccius 2002). Further work is needed to define the mechanism and cheese conditions needed for production.

Amino acid degradation plays a vital role in flavor development in cheese. A number of works in the past attempted to enhance free amino acid content in Cheddar cheese by direct addition of amino acids (Wallace and Fox 1997) and genetic modification of lactococci with increased aminopeptidase N activities (McGarry and Others 1994; Christensen and others 1995). But increased amino acid content in Cheddar did not affect the flavor development, which led Yvon and others (1998) to hypothesize that the rate-limiting factor in flavor biogenesis was not the release of amino acids but their subsequent conversion to aroma compounds. Yvon and others (1998) identified the transaminase acceptor α-ketoglutarate as the first limiting factor in the degradation of amino acids. Addition of α-ketoglutarate to Cheddar curd resulted in increased volatile components originating from branched chain and aromatic amino acids (Banks and others 2001). Results of a recent study showed that Cheddar cheese made using adjunct starter *Lactobacillus casei* (genetically modified to enhance expression of hydroxyl acid dehydrogenase, HADH) retarded the flavor development (Broadbent and others 2004). HADH catalyses conversion of α-keto acids to α-hydroxy acids, which has little or no importance from a flavor point of view. But it may still be possible to selectively suppress aromatic amino acid (Phe, Tyr, Trp)-derived off-flavor compounds by overexpression of an alternative HADH with more narrow specificity for aromatic amino acid-derived α-keto acids.

78.3 CHARACTERIZATION OF FLAVOR OF MILK AND MILK PRODUCTS

Flavor of milk and milk products is produced by a correct balance and concentration of a wide range of sapid and aromatic compounds ("Component balance theory" – Mulder 1952; Kosikowski and Mocquot 1958), see Tables 78.1 and 78.2 for a compilation of odorants found in milk and milk products. If a proper balance of components is not achieved, then undesirable or defective flavor occurs. The delicate but weak flavor of milk is caused by very low concentrations of numerous odorants (Badings 1991; Bendall 2001). Varying levels of heat treatment during processing of raw milk, for example, pasteurization, UHT-treatment or sterilization, has been shown to increase lipid oxidation products (see Table 78.1). When milk is severely heated, both flavor and color are affected. Volatile compounds that contribute particularly to sterilized milk are lipid oxidation products (mainly 2-alkanone : $C_{5,7,9,11}$) and several heterocyclic compounds, such as pyrazines, furans, pyrones, and other nonenzymatic browning/Maillard reaction products (e.g., maltol, furaneol, 2,5-dimethylpyrazine, and o-aminoacetophenone); see Badings (1991). Sweetened condensed milk also involves similar compounds in its volatile flavor profile (Shimoda and others 2001).

Flavor of fat enriched products like cream and butter are characterized, as one would expect, by lactones and lipid oxidation products (Table 78.1). Compounds like 2,3-butandione play an important role in the aroma of sour cream and sour cream butter. Aroma impact compounds of fresh and heat sweet cream butter were studied by Peterson and Reineccius (2003a,b).

Aroma of dried milk products, such as nonfat dry milk, with varying heat treatment, involves very similar compounds, as shown in Table 78.1. But the high heat-treated nonfat dried milk particularly has a strong aroma intensity caused by furaneol, butanoic acid, methional, o-aminoacetophenone, (*E*)-4,5-epoxy-(*E*)-2-decenal, sotolon, and vanillin. A stored sample of nonfat dried milk had particularly high odor intensities for methional and o-aminoacetophenone (see Table 78.1). Other dried products, such as rennet casein, an important food ingredient, particularly suffer from off-flavor. Results of aroma extract dilution analysis (AEDA) indicated o-aminoacetophenone to be a potent odorant; however, sensory descriptive sensory analysis of model aroma systems revealed that the typical odor of rennet casein was principally caused by hexanoic acid, indole, guaiacol, and p-cresol (Karagul-Yuceer and others 2003b). It is essential for rennet casein to be bland and free of any off-flavor for its use as a food ingredient. Diacetyl and acetaldehyde are important odorants in yogurt, and are produced by lactic acid bacteria as starter cultures. There are numerous other fermented milks produced around the world involving lactic acid bacteria and/or in some cases fairly complicated set of microflora in which a host of volatile compounds play a crucial role in flavor.

There has been extensive research on the flavor of cheeses, but despite this effort, only limited information is available on the chemistry of flavor of most cheese varieties and the flavor of none is characterized sufficiently to permit its reproduction by mixtures of pure compounds in a cheese model (Fox and others 1995; Parliment and McGorrin 2000; McGorrin 2001; Singh and others 2003a). Cheeses in many previous studies were simply analyzed for flavor by cheese graders. Such qualitative sensory data has limited use. More defined and analytical information using descriptive sensory and instrumental analysis is required. In the last couple of decades, a number of published works attempted to characterize the mechanism/enzymology of various reactions involved in generation of

TABLE 78.1 Aroma Compounds Identified in Milk and Milk Products (Compounds are Listed in the Order of Their Importance).

Milk (fresh)[a]	**Fresh butter**[c]	**Nonfat dry milk (low heat)**[e]	**Nonfat dry milk (high heat)**[e]	**Rennet casein**[g]
Ethyl hexanoate	δ-Decalactone	4-Hydroxy-2,5-dimethyl-(2H)-furan-3-one	4-Hydroxy-2,5-dimethyl-(2H)-furan-3-one	o-Aminoacetophenone
Ethyl butyrate	1-Hexen-3-one	Butanoic acid	Butanoic acid	2-Methoxyphenol
Dimethyl sulfone	δ-Dodecalactone	3-(Methylthio) propanal	3-(Methylthio) propanal	Hexanoic acid
Nonanal	1-Octen-3-one	o-Aminoacetophenone	o-Aminoacetophenone	Maltol
1-Octen-3-ol	Skatole	δ-Decalactone	(E)-4,5-Epoxy-(E)-2-decenal	4-Hydroxy-2,5-dimethyl-(2H)-furan-3-one
Indole	(Z)-6-Dodecen-γ-lactone	(E)-4,5-Epoxy-(E)-2-decenal	δ-Decalactone	4,5-Diemthyl-3-hydroxy-2(*5H*)-furanone
		Pentanoic acid	Pentanoic acid	Decanoic acid
Milk (pasteurized)[a]	**Heated butter**[c]	4,5-Diemthyl-3-hydroxy-2(*5H*)-furanone (Sotolon)	4,5-Diemthyl-3-hydroxy-2(5H)-furanone	(Z)-6-Dodecen-γ-lactone
Dimethyl sulfone	δ-Decalactone	3-Methoxy-4-hydroxybenzaldehyde (Vanillin)	3-Methoxy-4-hydroxybenzaldehyde phenyl acetic acid	Skatole
Hexanal	Skatole	2-Acetyl-1-pyrroline	Nonanal	Dodecanoic acid
Nonanal	3-(Methylthio) propanal	2-Acetyl-2-thiazoline	1-Octen-3-one	3-Methoxy-4-hydroxybenzaldehyde
1-Octen-3-ol	δ-Dodecalactone	Hexanoic acid	2-Acetyl-1-pyrroline	
Indole	4-Hydroxy-2,5-dimethyl-(2H)-furan-3-one (Furaneol)	Phenyl acetic acid	Hexanoic acid	**Liquid cheddar whey**[h]
Benzothiazole	1-Octen-3-one	Octanoic acid	Octanoic acid	2,3-Butanedione
δ-Decalactone	1-Hexen-3-one	Nonanal	(E)-2-Nonenal	Dimethyl sulfide
2-Tridecanone	(Z)-2-Nonenal	1-Octen-3-one		Hexanal
	(E)-2-Nonenal			
UHT milk[a]	(E,E)-2,4-Decadienal			
2-Heptanone	(E)-4,5-Epoxy-(E)-2-decenal			
2-Nonanone	γ-Octalactone			
2-Undecanone				
δ-Decalactone				
2-Tridecanone				
Dimethyl sulfone				
Benzothiazole				
Hexanal				
Indole				

(*Continued*)

TABLE 78.1 *Continued.*

Cream[b]	**Sour cream butter**[d]	**Nonfat dry milk (medium heat)**[e]	**Nonfat dry milk (stored)**[f]
γ-Decalactone	2,3-Butanedione	δ-Decalactone	3-(Methylthio) propanal
δ-Decalactone	δ-Decalactone	4-Hydroxy-2,5-dimethyl-(2H)-furan-3-one	o-Aminoacetophenone
δ-Dodecalactone	Butanoic acid	Butanoic acid	4-Hydroxy-2,5-dimethyl-(2H)-furan-3-one
(Z)-4-Heptenal	Hexanoic acid	3-(Methylthio) propanal	2-Methyl-3-hydroxy-*4H*-pyran-4-one
(E,E)-2,4-Nonadienal	(*Z*)-6-Dodecen-γ-lactone	o-Aminoacetophenone	Butanoic acid
		(E)-4,5-Epoxy-(E)-2-decenal	Pentanoic acid
		3-Methoxy-4-hydroxybenzaldehyde	Acetic acid
		2-Acetyl-1-pyrroline	Hexanoic acid
		2-Acetyl-2-thiazoline	Octanoic acid
		4,5-Diemthyl-3-hydroxy-2(5H)-furanone	Decanoic acid
		Hexanoic acid	Dodecanoic acid
		Phenyl acetic acid	p-Cresol
		γ-Dodecalactone	Skatole
		(E)-2-Undecenal	Dimethyl trisulfide
		(E,E)-2,4-Decadienal	(E,E)-2,4-Decadienal
			Furfuryl alcohol
			Phenyl acetic acid
			1-Octen-3-one

Abbreviations: GCO gas chromatography olfactometry.
AEDA aroma extract dilution analysis.
GC-MS gas chromatography-miss spectrometry.
DHS dynamic headspace.
Source: [a]Raw, pasteurized, and UHT treated milks analyzed by GCO/AEDA/GC-MS (Moio and others 1994).
[b]Cream data adapted from McGorrin (2001).
[c]Fresh and heated butter analyzed by GCO/AEDA/GC-MS (Budin and others 2001).
[d]Irish sour cream butter analyzed by GCO/AEDA/GC-MS (Schieberle and others 1993).
[e]Nonfat dried milks analyzed by GCO/AEDA/GC-MS (Karagul-Yuceer and others 2001, 2003a).
[f]Stored nonfat dried milks analyzed by GCO/AEDA/GC-MS (Karagul-Yuceer and others 2002).
[g]Rennet casein analyzed by GCO/AEDA/GC-MS (Karagul-Yuceer and others 2003b).
[h]Liquid Cheddar whey analyzed by DHS/GC-MS (Carunchia-Whetstine and others 2003a).

TABLE 78.2 Aroma Compounds Identified in Different Fermented Dairy Products (Compounds are Listed in the Order of Their Importance).

Mozzarella (water buffalo milk)[a]	**Blue-type**[c]	**Swiss Gruyere**[e]	**Mild Cheddar**[f]	**Sharp Cheddar** (British farmhouse)[g]	**Parmigiano Reggiano**[h]
1-Octen-3-ol	2,3-Butanedione	2/3-Methyl butanal	Furaneol	2-Isopropyl-3-methoxypyrazine	3-Methyl butanal
Nonanal	2-Methyl propanal	3-(Methylthio) propanal	(*E*)-2-Nonenal	3-(Methylthio) propanal	2-Methyl propanal
Indole	3-Methyl butanal	Dimethyl trisulfide	2,3-Butanedione	p-Cresol	2-Methyl butanal
3-Hydroxy-2-pentanone	Ethyl butanoate	Phenyl acetaldehyde	(*Z*)-4-Heptenal	δ-Dodecalactone	Dimethyl trisulfide
3-Methyl-2-buten-1-ol	Ethyl hexanoate	2-Ethyl-3,5-dimethylpyrazine	3-(Methylthio) propanal	Butanoic acid	2,3-Butanedione
2-Octanone	3-(Methylthio) propanal	2,3-diethyl-5-methylpyrazine	1-Octen-3-one	Isovaleric acid	3-(Methylthio) propanal
2-Hydroxy-3-pentanone	Dimethyl trisulfide	Methanethiol	2-Acetyl-2-thiazoline	2-Phenylethanol	Phenyl acetaldehyde
Heptanal	2-Heptanone	Acetic acid	Dimethyl trisulfide	Ethyl octanoate	Ethyl butanoate
Mozzarella (Bovine milk)[a]	2-Nonanone	Propanoic acid	(*Z*)-1,5-Octadien-3-one	Acetic acid	Ethyl hexanoate
Ethyl-3-methyl butanoate		Butanoic acid	(*Z*)-2-Nonenal	β-Damascenone	Ethyl octanoate
Ethyl isobutanoate	**Camembert**[d]	Isovaleric acid	Ethyl butanoate	Octanoic acid	Acetic acid
2/3-Methyl-1-butanol	2,3-Butanedione	Phenyl acetic acid	Hexanal	4,5-Dimethyl-3-hydroxy-2(*5H*)-furanone	Butyric acid
Phenyl acetaldehyde	3-Methyl butanal		2-Isobutyl-3-methoxypyrazine	(sotolon)	Hexanoic acid

(*Continued*)

TABLE 78.2 *Continued*.

Ethyl hexanoate	3-(Methylthio) propanal	*trans*-4,5-Epoxy-2-(*E*)-decenal	Phenyl acetic acid	Octanoic acid
Ethyl butanoate	1-Octen-3-ol	2-Nonanone	Ethyl butanoate	
Nonanal	1-Octen-3-one	2-Isopropyl-3-methoxypyrazine	Ethyl hexanoate	
1-Octen-3-ol	Phenyl ethyl acetate	Decanal	Dimethyl trisulfide	
	2-Undecanone	2/3-Methyl butanal	Phenyl acetaldehyde	
Yoghurt[b]	δ-Decalactone	Ethyl octanoate	Pentanoic acid	
2,3-Butanedione	Methanethiol	1-Hexen-3-one	Guaiacol	
Acetaldehyde	Dimethyl sulfide	Methyl propanal	γ-Decalactone	
Dimethyl sulfide	Acetaldehyde	Ethyl hexanoate	δ-Decalactone	
Benzaldehyde	Hexanal	Homofuraneol	1-Octen-3-one	
2,3-Pentanedione	Dimethyl trisulfide	Butyric acid	2-Acetylpyrazine	
	Butyric acid		2-Isobutyl-3-methoxypyrazine	
	Isovaleric acid		Linalool	
			(*E,Z*)-2,6-Nonadienal	
			Geosmin	
			Furaneol	

Source: [a]Mozzarella cheese analyzed by GCO/AEDA/GC-MS (Moio and others 1993).
[b]Yoghurt data adapted from McGorrin (2001).
[c]Blue cheese analyzed by GCO/AEDA/GC-MS (Qian and others 2002).
[d]Camembert cheese analyzed by GCO/AEDA/AECA/GC-MS and GCO-H (Kubickova and Grosch 1997).
[e]Swiss Gruyere cheese analyzed by GCO/AEDA/GC-MS and DH-GC-MS (Rychlik and Bosset 2001a,b).
[f]Mild Cheddar cheese analyzed by GCO/AEDA/GC-MS and GCO-DHDA/GC-MS (Zehentbauer and Reineccius 2002).
[g]British farmhouse Cheddar cheese analyzed by GCO/AEDA/GC-MS (Suriyaphan and others 2001b).
[h]Parmigiano Reggiano cheese analyzed by DH-GC-MS and OAV (Qian and Reineccius 2002).

volatiles in cheese. Only recent work in the last decade has attempted to study cheese flavor in detail.

In recent years, key odorants in a number of cheese varieties have been characterized by gas chromatography olfactometry/aroma extract dilution analysis (GCO/AEDA). Mozzarella cheese produced using buffalo and cow milk was found to have very different volatile compounds in their aroma profiles (Table 78.2). Odorants in surface mold ripened cheeses like Camembert were studied in detailed by Kubickova and Grosch (1997, 1998a,b). Aroma compounds were analyzed by GCO using both aroma extract dilution and aroma extract concentration analysis. Compounds like 1-octen-3-ol and the corresponding ketone were found to be responsible for the mushroom/musty aroma note of Camembert. Kubickova and Grosch (1998a) incorporated key odorants identified in Camembert in a model cheese, which was found to be close to the genuine Camembert. Origins and properties of compounds involved in the flavor of surface ripened cheeses were reviewed by Molimard and Spinnler (1996). Key odorants in Swiss Emmentaler aroma profile were methional, furaneol, ethyl furaneol, diacetyl, 3-methyl butanal, and esters (Preininger and Grosch 1994). Cheese models composed of methional, furaneol, ethyl furaneol, acetic acid, propanoic acid, lactic acid, succinic acid, glutamic acid, sodium, potassium, calcium, magnesium, ammonium, phosphate, and chloride were judged to match the flavor of Swiss Emmentaler cheese very well (Preininger and others 1996). The flavor of typical Swiss Gruyere cheese and a Gruyere sample exhibiting a potato like off-flavor were characterized by Rychlik and Bosset (2001a,b). Odorants like methional, 2-ethyl-3,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine were the probable source of potato-like off-flavor in Gruyere.

Muenster cheese made in the United States, using *Streptococcus thermophilus* as culture and no surface smear, was described by cooked/milky, whey, milk fat/lactone, sour, and salty notes using descriptive sensory analysis. The use of dynamic headspace dilution analysis (DHDA) methodology, previously described by Cadwallader and Baek (1998), showed that the most aromatic compounds in the headspace of Muenster were 2,3-butanedione, dimethyl sulfide, dimethyl disulfide, 2/3-methyl butanal, and 2-acetyl-2-thiazoline (Singh and others 2003b).

4-Methyl octanoic and 4-ethyl octanoic acids were found to impart waxy/crayon odors to fresh goat cheese (Carunchia-Whetstine and others 2003b). Numerous other odor active compounds were identified in goat cheese and assessed by sensory analysis of model cheeses for their specific role in the overall aroma.

Flavor of Cheddar cheese is by far the most widely studied. It is generally accepted that the flavor quality of Cheddar cheese in the marketplace today differs considerably from that manufactured before the wide use of pasteurization, microbial rennets, and other modern manufacturing practices (Dunn and Lindsay 1985). Much of the differences between traditional and contemporary Cheddar flavors probably should be attributed to current marketing of bland-flavored young cheeses. However, even longer aged cheeses are frequently criticized for a lack of adequate Cheddar-type flavor.

The significance of sulfur-compounds, such as H_2S, methanethiol, and dimethyl sulfide, in Cheddar cheese was shown by Manning and Robinson (1973). The compounds with low vapor pressure/high boiling points in the distillate, such as 2,3-butanedione, methyl ketones, and volatile fatty acids were also considered to play an important role in Cheddar flavor. Analysis of Cheddar headspace volatiles also reconfirmed the important role played by H_2S, methanethiol, and dimethyl sulfide in flavor (Manning and Price 1977; Manning and Moore 1979; Price and Manning 1983).

In order to evaluate important odorants, GCO/AEDA was first applied to Cheddar cheese by Christensen and Reineccius (1995). The components found to have the highest potency (dilution factor) in a 3-year-old Cheddar cheese were ethyl acetate, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, α-pinene, ethyl butyrate, ethyl caproate, 1-octen-3-one, acetic acid, methional, propionic acid, butyric acid, valeric acid, caproic acid, capric acid, and lauric acid. The authors pointed out that the technique did not allow the determination of the most volatile odor fraction, which included hydrogen sulfide, acetaldehyde, and methanethiol. Descriptive sensory analysis was not conducted on the cheese used in the study, which limited conclusions about the role of individual compounds on specific cheese flavors. Based on these results, a subsequent sensory study using a concept matching technique was conducted. Dacremont and Vickers (1994) found that a recognizable Cheddar aroma was produced by a mixture of 2,3-butanedione, methional, and butyric acid. However, the authors also indicated a possible contribution of other aroma compounds that were not commercially available at that time.

Milo and Reineccius (1997) applied both traditional high vacuum isolation/aroma extract dilution analysis (AEDA) and static headspace-olfactometry (GCO-H) to further study the aroma of a regular and a low-fat Cheddar cheese (see Table 78.2). After the quantification and calculation of respective odor activity values, based on sensory thresholds in oil and water, they suggested acetic acid, butyric acid, methional, 2,3-butanedione, and homofuraneol as the primary odorants responsible for the pleasant mild aroma of Cheddar cheese. In addition to the above mentioned compounds, the contribution of highly volatile sulfur compounds, such as methanethiol and dimethyl sulfide, to nasal perception of Cheddar cheese was quite obvious on the basis of GCO analysis of static headspace samples. The authors further hypothesized that the meaty-brothy odor characteristic of low-fat Cheddar was caused by high concentrations of methional, furaneol, and especially homofuraneol. The furaneol-type odorants are known to be produced by certain strains of lactobacilli (Preininger 1995; cited from Milo and Reineccius 1997). While the mixture of these volatile compounds in a model cheese matrix had Cheddar aroma, attribute profiling described it as lacking in sour, moldy, and sulfurous notes relative to the real cheese. Also the overall odor was described as weak. This discrepancy in sensory character between the aromatized model and real cheese was partially caused by aroma-matrix interactions, which resulted in quantitative errors (Wang and Reineccius 1998).

A comparison of the volatile compositions of full- and reduced-fat Cheddar showed that the level of methanethiol in the cheese is highly correlated with the flavor grade. This observation may indicate that the lack of aroma in reduced-fat Cheddar is due to lack of methanethiol. However, a combination of methanethiol and decanoic acid or butanoic acid in all cheeses gave a better correlation with Cheddar flavor than methanethiol alone (Dimos and others 1996). Addition of methanethiol to a bland slurry of reduced-fat Cheddar produced a strong Cheddar aroma (Urbach 1997).

The use of DHDA methodology has suggested additional volatiles as being important to Cheddar cheese aroma as compared to gas chromatography olfactometry-static headspace (GCO-H) and solvent extraction/AEDA (Zehentbauer and Reineccius 2002), (see Table 78.2) Results of DHDA showed that in addition to the odorants previously identified by AEDA and GCO-H, (Z)-4-heptenal, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, (Z)-1,5-octadiene-3-one, and (E)/(Z)-2-nonenal, which have been underestimated or not even perceived during AEDA, may also contribute to the overall aroma of Cheddar cheese.

The volatile aroma components of two sharp Cheddar cheeses of British Farmhouse origin, made using raw milk and ripened for at least 1 year, were analyzed by AEDA (Suriyaphan and others 2001b; Table 78.2). Descriptive sensory analysis of these cheeses was also conducted. Key flavors in sharp Cheddar cheeses were "barnyard" and "earthy." Following instrumental analysis, model system addition was used to confirm compounds responsible for specific flavor notes. p-Cresol was mainly responsible for a "cowy-barny" note, whereas an intense "soil-like" note was due to 2-isopropyl-3-methoxypyrazine. At much lower odor intensity, 2-isobutyl-3-methoxypyrazine contributed a "bell pepper-like" note. Direct addition of p-cresol (>100 ppb) or 2-isopropyl-3-methoxypyrazine (>3 ppb) in a mild domestic Cheddar cheese resulted in increases in intensities of cowy/phenolic and earthy/bell pepper aroma notes. Additionally, within the same wedge of cheese, the concentrations of p-cresol and 2-isopropyl-3-methoxypyrazine were lower at the center than at the rind.

It is important to note that in each of the studies mentioned previously, different Cheddar cheeses of different ages, microflora, and biochemistry were studied. Cheddar cheese encompasses a wide category and there are numerous potential flavor profiles. Thus, to elucidate Cheddar cheese flavor is a large task and descriptive sensory analysis should be conducted in conjunction with any instrumental study to provide clarification.

78.3.1 Chemical Anchors for Specific Flavors or Off-Flavors in Milk and Milk Products

In addition to the characteristic desirable flavors, dairy products frequently suffer from specific flavor defects. While desirable flavor has been difficult to define in chemical and sensory terms since consumers vary in preference and definition of dairy products flavor, the specific cause(s) of many of these specific flavor or off-flavor notes have been established more or less definitively. This section presents an overview on chemicals responsible for the specific flavor/off-flavor notes in dairy products.

It is important to note that in the beginning of the development of flavor defects, or before the aroma detection threshold of certain off-flavors is reached, the fluid milk may show a poor/flat flavor that lacks freshness (Badings 1991). Off-flavors in milk could develop via several different mechanisms outlined below (see Table 78.3 for causative chemical/mechanism involved):

- Off-flavor transferred to milk during lactation
  - Feed off-flavors (e.g., from green forages, silage, etc.) consumed by animal a few hour before milking
  - Weed off-flavor (e.g., volatiles formed from certain weeds during digestion)
- Cowy flavors (related to ketosis and acetonemia in cattle resulting in increased concentration of acetone)
- Stable off-flavors (e.g., transferred via respiratory system from stable/feed to cow)
- Lipid oxidation – most widely implicated in the defects in milk
- Microbial action
- Contamination (e.g., compounds from sanitizers)
- Thermal abuse

TABLE 78.3 Specific Flavor and Off-Flavor Compounds in Cheeses.

Defect	Chemicals	Mechanism of Formation	Reference
Processed milk			
Oxidized	Heptanal, octanal, nonanal, 2-octenal, 2-nonenal	Lipid oxidation, light abuse, Cu oxidation	Forss and others (1955a,b)
Malty	2/3-methyl butanal	Microbial, enzymatic, Strecker degradation	Morgan (1970a,b)
Metallic	1-Octen-3-one	Lipid oxidation	Day and others (1963)
Fruity	Ethyl butanoate, ethyl hexanoate	Microbial, enzymatic	Wellnitz-Ruen and others (1982) Whitfield and others (2000)
Rancid	FFAs (C4-10)	Microbial, enzymatic, sanitizer	Azzara and Campbell (1992) Marsili (2000)
Oxidized	Hexanal, 1-octen-3-one	Lipid oxidation, light abuse, Cu oxidation	Cadwallader and Howard (1998) Marsili and Miller (1998)
Oxidized (general)	Aldehydes and ketones	Lipid oxidation	Badings (1991)
Oxidized (fatty/fried)	2-Alkenals (C7-C10), 2,4-alkadienals (C7, C10)	Lipid oxidation	Badings (1991)
Oxidized (green/cucumber)	(Z)-3-Hexenal, (E,Z)-2,6-nonadienal	Lipid oxidation	Badings (1991)
Oxidized (tallowy)	2-Alkenals (C7-C10), alkanals	Lipid oxidation	Badings (1991)
Oxidized (fishy)	2,4,7-Decatrienal	Lipid oxidation	
Cooked flavor	H2S, methanethiol, dimethylsulfide and other sulfide	Thermally induced	Boelrijk and de Jong (2003)
	Maltol, furans, pyrazines and other Maillard products	Thermally induced	Badings (1991)
Weed taints	Indole, skatole, mercaptans, sulfides, nitriles, thiocyanates	Metabolites of weed	Badings (1991)
	Skatole (fecal odor)	Metabolites of weed	Park and others (1969)
	Benzylthiol, benzyl methyl sulfide (burnt odor)	Metabolites of weed	Park and others (1969)
Feed flavors	Dimethyl sulfide, acetone, butanone, isopropanol, ethanol, propanol	Metabolites of feed and silage	Badings (1991)
Light induced	Dimethyl disulfide	Light induced degradation (singlet oxygen oxidation) of methionine	Jung and others (1998)
Heat abuse, cooked	2-Pentanone, 2-heptanone, 2-nonanone	Thermally induced, microbial	Marsili and Miller (2003)

(*Continued*)

TABLE 78.3 *Continued.*

Defect	Chemicals	Mechanism of Formation	Reference
Sour cream buttermilk			
Metallic	(*E,Z*)-2,6-Nonadien-1-ol	Lipid oxidation, microbial, enzymatic	Helier and Schieberle (1997a,b)
Butter			
Green or yogurt-like	Acetaldehyde	Microbial, enzymatic	Lindsay and others (1965)
Metallic	1,5-Octadiene-2-one	Lipid oxidation	Swododa and Peers (1977)
Yogurt			
Smoky or phenolic aroma	Guaiacol (defect found in vanilla flavored products)	Microbial	Whitfield (1998)
Butter oil			
Card board	(*E*)-/(*Z*)-2-Nonenal	Lipid oxidation	Grosch and others (1994)
Skim milk powder			
Stale	o-Aminoacetophenone	–	Parks and others (1964)
Stale	2-Furaldehyde, 2-furfuryl butyrate, alkylpyrazine, N-ethyl-2-formylpyrrole	Non-enzymatic browning	Ferretti and Flanagan (1972)
Cow house-like	β-Ionone, benzothiazole, tetradecanal	–	Shiratsuchi and others (1994)
Papery/card board-like	(E,E)-2,4-Nonadienal, (E,E)-2,4-decadienal	Lipid oxidation	Karagul-Yuceer and others (2003a)
Sterilized concentrated milk			
Stale	o-Aminoacetophenone	–	Arnold and others (1966)
Swiss Gruyere			
Potato-like aroma	Methional	Microbial, enzymatic	Rychlik and Bosset (2001a,b)
Gruyere de Comte			
Potato-like aroma	3-Methoxy-2-propylpyridine	Microbial	Dumont and others (1975)
Smear-coated cheese			
Potato-like aroma	2-Methoxy-3-isopropylpyrazine	Microbial	Dumont and others (1983)
Feta			
Kerosene-like	*trans*-1,3-Pentadiene	Microbial, enzymatic	Horwood and others (1981)
Goat cheese			
Waxy/crayon	4-methyl octanoic acid, 4-ethyl octanoic acid	Milk, enzymatic	Carunchia-Whetstine and others (2003b)
Cheddar cheese			
Unclean-off-flavor	2-Methyl propanoic acid, 3-methyl butanoic acid	Microbial, enzymatic	Nakae and Elliot, (1965a,b)

(*Continued*)

TABLE 78.3 *Continued.*

Defect	Chemicals	Mechanism of Formation	Reference
Catty flavor	2-mercapto-2-methylpentan-4-one	Reaction of mesityl oxide (contaminant) and sulfide	Badings (1967), Spencer (1969a,b) Drake and others (2001, 2002)
Fruitiness	Ethyl butanoate, ethyl hexanoate, ethyl octanoate	Microbial, enzymatic	Bills and others (1965), Morgan (1970b)
Floral/rose-like	Phenyl ethanol, Phenyl acetaldehyde	Microbial, enzymatic	Dunn and Lindsay (1985)
Unclean, utensil-like p-Cresol	(Off-flavor enhanced by FFAs)	Microbial, enzymatic	Dunn and Lindsay (1985)
Unclean (dull harsh)	2/3-Methyl butanal, 2-methyl propanal	Microbial, enzymatic, Strecker degradation	Dunn and Lindsay (1985)
Rosy/floral	Phenylacetaldehyde, phenylacetic acid	Microbial, enzymatic	Carunchia-Whetstine and others (2004)
Yeasty flavor	Ethanol, ethyl acetate, ethyl butanoate	Microbial, enzymatic	Horwood and others (1987)
Mayonnaise/bread-like	(E,E)/(E,Z)-2,4-Decadienal	Microbial, Lipid oxidation, enzymatic	Suriyaphan and others (1999, 2001a)
Cowy/barny-flavors	p-Cresol	Microbial, enzymatic	Suriyaphan and others (2001b)
Earthy/bell pepper	2-isopropyl-3-methoxypyrazine	Microbial, Maillard reaction	Suriyaphan and others (2001b)
Brothy flavor	3-(Methylthio)-propanal, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, 2-ethyl-4-hydroxy-5-methyl-3(*2H*)-furanone, 2-Methyl-3-furanthiol (its dimerized form)	Microbial, enzymatic, Maillard reaction	Singh and others (2004a)
Nutty flavor	2-Methyl propanal, 2/3-Methyl butanal	Microbial, enzymatic, Strecker degradation	Avsar and others (2004)

Some of the mechanisms of off-flavor development, in particular lipid oxidation and microbial metabolites, have also been responsible for the development of a whole variety of off-flavors in milk and milk products (see Table 78.3). Specific flavors like nutty or brothy flavors were also characterized in Cheddar cheese (Table 78.3), which may or may not be considered as an off-flavor depending on the consumer preference.

78.3.2 Taste Compounds in Milk and Milk Products

Research on taste compounds in dairy products is fairly limited but there seems to be an increasing interest in this area in recent years. In food systems such as milk and milk products, study of taste sensation/compounds in isolation or devoid of contribution from volatile aroma compounds is difficult due to complex nature, in terms of both number of food constituents and their competing/synergistic effect on taste and/or aroma. Compounds which contribute to taste of milk and milk products can originate from three possible sources:

1. Naturally found in milk, for example, lactose,
2. Added/produced during the manufacturing process, for example, NaCl, lactic acid,
3. Produced by many biochemical reactions occurring during fermentation.

Table 78.4 lists a number of compounds and their taste contribution, present in dairy products. Important details available in the literature on taste compounds are summarized below:

- The main taste compounds in milk are lactose (approximately 0.3 times as sweet as sucrose) and the dissolved salts, which cause a sweet and salty taste, respectively. The sweet taste dominates, whereas salty taste is prevalent if the Ca/lactose ratio is high as in the case of mastitic milk. The casein reportedly somewhat masks the sweet taste of lactose in milk (Walstra and others 1999). Lactose hydrolyzed milk and whey have sweeter taste than regular pasteurized milk.
- A chalky taste is noticed in the high heat-treated or UHT treated milks. This may be the result of precipitation of colloidal calcium phosphate/change in mineral balance.
- Sodium chloride is an important contributor to the taste of cheeses. The apparent saltiness of cheese increases with maturity, increased NaCl concentration, and decreasing pH (McSweeney 1997).
- The principal acid in fermented milk and cheese is lactic acid. The concentration of lactic acid, and also the pH, varies considerably with:
  - The type of fermented dairy products
  - Initial production by the starter culture
  - Extent of loss in whey
  - Its metabolism by the non-starter microflora.
- Several other acids, for example, acetic, propanoic, and C_{4-10}, also contribute to sour/soapy taste but they predominantly contribute to the aroma. Some of the characteristic taste (sour, sweet, salty) compounds of Emmentaler (Swiss) cheese were acetic acid, propanoic acid, lactic acid, succinic acid glutamic acid, each in free form and/or as ammonium, sodium, potassium, magnesium, and calcium salts as well as corresponding chlorides and phosphates (Warmke and others 1996). Magnesium and calcium propionate mainly caused the sweetish note in the taste profile of Emmentaler cheese.
- Casein is hydrolyzed to varying degrees depending on the type of fermented milk and cheese, resulting in the production of peptides and free amino acids. The precise role of the intermediate to small molecular weight peptides is not clear; however it is generally accepted to play an important role in the background taste of cheese (Fox and others 1994). Several peptides were identified in different types of cheeses as bitter, see Table 78.4 for a list of bitter peptides. An interesting relationship was established by Ney (1981) between the average hydrophobicity (Q) of a peptide, as measured by the hydrophobicity of amino acid side chains determined by Tanford (1962), and bitterness. The peptides with Q-values >1400 cal/mol/residue and molecular weights up to 6000 Da (molecules >6000 Da are likely to be too large to interact with the taste receptors) taste bitter, and no bitterness occurs when Q is <1300 cal/mol/residue. Peptide β-CN f193–209, identified in both Cheddar and Gouda, determined to be bitter by detailed sensory analysis (Singh and others 2004b). This peptide with Q value 1839 cal/mol/residue and molecular weight 1882.51 Da was also classified

TABLE 78.4 Bitter Peptides Identified in Cheddar Cheese.

Peptide	Hydrophobicity	Sequence	Type of Cheese and Reference
α_{s1}-CN f1-7	1771.0	H.Arg.Pro.Lys.His.Pro.Ile.Lys.OH	Cheddar; Lee and others (1996)
α_{s1}-CN f1-13	1363.0	H.Arg.Pro.Lys.His.Pro. Ile.Lys.His.Gln.Gly.Leu.Pro.Gln.OH	Cheddar; Lee and others (1996)
α_{s1}-CN f11-14	1367.0	H.Leu.Pro.Gln.Glu.OH	Cheddar; Lee and others (1996)
α_{s1}-CN f14-17	1162.5	H.Glu.Val.Leu.Asn.OH	Cheddar; Hodges and others (1972), Richardson and Creamer (1973), Hamilton and others (1974)
α_{s1}-CN f17-21	1074.0	H.Asn.Glu.Asn.Leu.Leu.OH	Cheddar; Hodges and others (1972), Richardson and Creamer (1973), Hamilton and others (1974)
α_{s1}-CN f26-32	1930.0	H.Ala.Pro.Phe.Pro.Glu.Val.Phe.OH	Cheddar; Richardson and Creamer (1973)
α_{s1}-CN f26-33	1688.8	H.Ala.Pro.Phe.Pro.Glu.Val.Phe.Gly.OH	Cheddar; Hodges and others (1972), Hamilton and others (1974)
α_{s1}-CN f198-199	2710.0	H.Leu.Trp.OH	Alpkäse; Guigoz and Solms (1974)
α_{s2}-CN f191-197	2010.0	H.Lys.Pro.Trp.Ile.Gln.Pro.Lys.OH	Cheddar; Lee and others (1996)
β-CN f8-16	1390.0	H.Val.Pro.Gly.Glu.Ile.Val.Glu.Ser(P).Leu.OH	Cheddar; Lee and others (1996)
β-CN f46-67	1580.5	H.Gln.Asp.Lys.Ile.His.Pro.Phe.Ala.Gln.Thr.Gln.Ser.Leu.- Val.Tyr.Pro.Phe.Pro.Gly.Pro.Ile.(Pro/His).OH	Cheddar; Richardson and Creamer (1973), Hamilton and others (1974)
β-CN f61-69	1792.2	H.Pro.Phe.Pro.Gly.Pro. Ile.Pro.Asn.Ser.OH	Butterkäse; Huber and Klostermeyer (1974)
β-CN f46-84	1508.5	H.Gln.Asp.Lys.Ile.His.Pro.Phe.Ala.Gln.Thr.Gln.Ser.Leu.- Val.Tyr.Pro.Phe.Pro.Gly.Pro.Ile.(Pro/His).Asn.Ser.- Leu.Pro.Gln. Asn.Ile.Pro.Pro.Leu.Thr.Gln.Thr.Pro.Val.Val.Val.OH	Cheddar; Hamilton and others (1974)
β-CN f84-89	1983.3	H.Val.Pro.Pro.Phe.Leu.Gln.OH	Gouda; Visser and others (1983)
β-CN f193-209	1762.4	H.Tyr.Gln.Glu.Pro.Val.Leu.Gly.Pro.Val.Arg.Gly.Pro.Phe. Pro.Ile.Ile.Val.OH	Cheddar; Kelly (1993), Broadbent and others (1998), Soeryapranata and others (2002a,b), Singh and others (2004a) Gouda; Visser and others (1983)
β-CN f193-208	1766.9	H.Tyr.Gln.Glu.Pro.Val.Leu.Gly.Pro.Val.Arg.Gly.Pro. Phe.Pro.Ile.Ile.OH	Gouda; Visser and others (1983)
β-CN f193-207	1686.7	H.Tyr.Gln.Glu.Pro.Val.Leu.Gly.Pro.Val.Arg.Gly.Pro. Phe.Pro.Ile.OH	Gouda; Visser and others (1983)

TABLE 78.5 Taste Compounds in Milk and Milk Products.

Compounds	Taste/Other Complex Sensation	Products
Lactose	Sweet	Milk, concentrated/evaporated milks, dried milk powders
Sucrose	Sweet	Sweetened yogurt, ice creams, sweetened condensed/ evaporated milks
Lactic acid	Sour	Fermented milks/cream, cheeses
Acetic/propanoic acid	Sour	Fermented milks, cheeses
Ca/Mg salt of propanoic acid	Sweet	Cheeses
Peptides	Mostly bland, some can be bitter, sour or umami	Milk, Fermented milks, cheeses
Amino acids Gly, Ala, ser, Thr	Sweet	Cheeses
Glu, Asp, Gln, Asn	Sour, umami	Cheeses
His	Sour (?)	Cheeses
Pro, Lys	Sweet, bitter3	
Leu, Val, Ile, Arg, Phe, Tyr	Bitter	Cheeses
TrpNaCl	Salty	Cheeses
Ethanol	Slightly sweet, cooling/drying sensation	Fermented milks (e.g., kefir, koumiss)
CO_2	Sour, fresh, cooling sensation	Fermented milks (e.g., kefir, koumiss)

as potentially bitter in the Q value model proposed by Ney (1981). Amino acids are also known to elicit different taste, (see Table 78.5).

Recently, a number of workers studied taste active compounds in Camembert (Engel and others 2001a,b,c), Cheddar (Yang and Vickers 2004), Comte (Salles and others 1995), Goat (Engel and others 2000a,b, 2002; Salles and others 2002), and Emmentaler cheeses (Warmke and others 1996). The topic of cheese taste was also reviewed by McSweeney (1997).

78.4 CONCLUSIONS

A concerted series of chemical and biochemical reactions are involved in the formation of dairy flavor and off-flavor compounds. General chemical/biochemical pathways, that is, (1) heat-induced changes, (2) lipid oxidation, (3) glycolysis, (4) lipolysis, and (5) proteolysis, involved in the degradation of milk constituents are now fairly well characterized. Recent works on the enzymology and genetic manipulation of the starter and nonstarter lactic acid bacteria have helped in the better understanding of further catabolic modification of the products of primary degradation pathways. This has lead to immense progress in understanding of the fermented dairy products flavor chemistry. So far a large number of volatile compounds have been characterized from various types of cheeses but still it is not possible to duplicate cheese flavor by pure chemicals in model systems. But there is now good understanding on the causes of bitterness and specific flavors/off-flavors in dairy products.

Developments in sensory and instrumental methodologies in flavor analysis have been of immense help in the understanding of dairy products flavor chemistry. Further work on

the characterization of flavor compounds, volatile flavor compounds-matrix interaction mechanisms and flavor release mechanisms are needed to fully elucidate dairy products flavor. The better understanding of flavor chemistry will be useful in the development of new technology/mechanisms for the effective control and acceleration of the ripening process in cheese.

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Section XV

Poultry Products

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

79

Poultry Marination

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79.1 Introduction 752
79.2 Raw Materials 753
79.2.1 Water Holding Capacity 755
79.2.2 Texture 755
79.2.3 Color 756
79.2.4 Flavor 757
79.3 Functional Ingredients 757
79.3.1 Salt 758
79.3.2 Phosphates 759
79.3.3 Species and Flavorings 760
79.3.4 Flavor Enhancers 762
79.3.5 Sweeteners 763
79.3.6 Binders and Extenders 764
79.3.7 Acidulants 764
79.3.8 Antioxidants 765
79.3.9 Antimicrobials 766
79.4 Marinating Systems 767
79.5 Packaging and Labeling 768
79.6 Microbial Considerations 769
79.7 Conclusion and Future Trends 769
References 769

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

79.1 INTRODUCTION

The poultry industry has had a remarkable growth all over the world during the last three decades. According to the National Chicken Council, per capita consumption of poultry in the United States has increased 120% since 1975 and it is expected to continue this way. Meanwhile, red meat and fish consumption had small variations during the same period (Fig. 79.1) due to changes in consumer life style and food preferences even with the recent fear of an avian influenza outbreak that has already infected millions of birds throughout Asia and parts of Europe. For instance, increases in broiler meat consumption from 2005 to 2006 were forecasted for Brazil (4%), China (3%), India (16%), Mexico (5%), Russia (7%), and the United States (3%) by the Foreign Agricultural Service (FAS/USDA 2005). In order to maintain this continuous growth in poultry consumption, producers and processors necessitate products that meet consumer demands for nutritious, safe, affordable, and convenient foods, which cook faster and require less preparation time. Nowadays, further processed poultry such as boneless and marinated items represent 50% of the total broiler market in the United States (Fig. 79.2); therefore the poultry industry and researchers should build up advanced processing technologies to produce consumer-oriented products, and to solve critical issues such as color, flavor, texture, and microbial safety related to the quality of poultry meat.

Marinating dates from the early 17th century, when meat was cured by immersion in brine (*aqua marina*) for preservation or mask off-flavors; a variety of liquids such as vinegar, lemon, wine, and oils, added with salt and spices have been used to marinate according to the cuisine and consumer preferences. Currently, this term involves an industrial process where meat, fish or poultry become tenderized and/or absorb flavor by the addition of water, flavors, and functional ingredients accomplished by immersion, injection, tumbling, or massaging. The marinating solution or marinade may be formulated with salt, phosphates, seasonings, carbohydrates, vegetable and dairy proteins, acids, antimicrobials, and antioxidants, among other ingredients (Brant 2003). However,

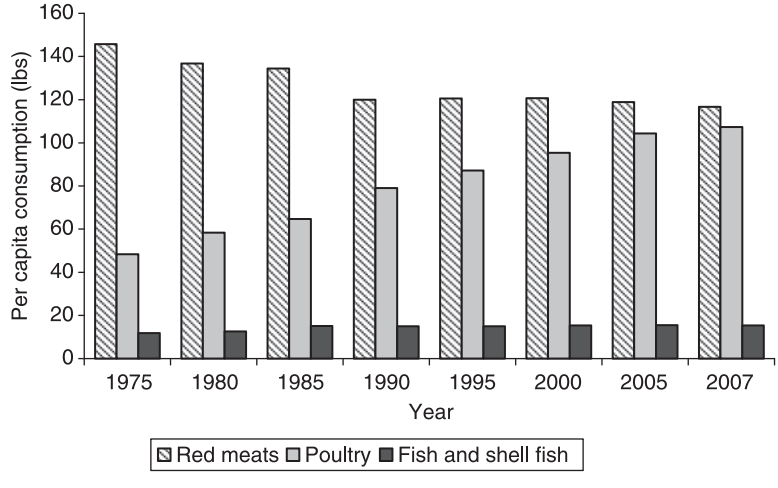


Figure 79.1 *Per capita consumption of poultry, livestock, fish and shellfish. (Red meats include beef, pork, veal, mutton, lamb, but exclude edible offals. Source: National Chicken Council.*

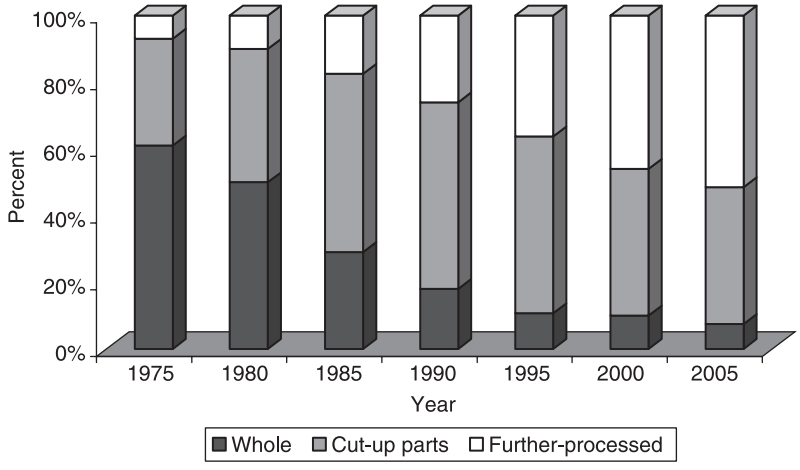


Figure 79.2 *Broiler presentation in market (data estimated for 2005). Source: National Chicken Council.*

it is necessary to understand how this process affects raw materials, as well as the functionality of ingredients, packaging materials, and also good manufacturing practices to create a high quality, safe, value added, tender, and flavorful marinated poultry.

79.2 RAW MATERIALS

Marinated poultry items include whole birds, cut-up parts, boneless or skin-less parts, and chopped or reformed meats. Meat must be inspected and achieve sanitary specifications, in the United States a representative of USDA's Food Safety Inspection Services (FSIS) ensures compliance of regulatory requirements. Meat must be free of off-odors (rancidity, sulfide, oily, etc.), and defects (discoloration, blood clots, excessive fat, soft like texture, etc.), with no evidence of mishandling, dehydration, or freezing and thawing, or apparent microbial growth, along with a characteristic color. Poultry may be fresh chilled or frozen, but chilled meat should not be above 40°F (4.4°C), and frozen meat should be below 0°F (−17.8°C) when received; the higher receiving temperature means a risk for pathogens and accelerates spoilage. In addition, temperature must not exceed 55°F (12.8°C) at any time during the preparation and processing according to the regulation of the U.S. Agricultural Marketing Service (AMS/USDA). Furthermore, the presence of foreign materials, chemical residues, insects, or other contaminants make raw materials inedible and inappropriate to be processed.

Poultry meat contains about 20% protein that according to their solubility and location are classified as myofibrillar, sarcoplasmic, and stroma. Miofibrillar proteins constitute 55% of total protein, are insoluble in water, and include more than 20 different proteins divided into three groups according to their function as: contractile (responsible for muscle contraction), regulatory (direct contraction), and citoskeletal proteins (give support and maintain muscle ultra structure). The contractile proteins, myosin and actin compose the actomyosin complex during muscle contraction and post mortem, also

they are the main proteins responsible for meat quality and functionality. On the other hand, sarcoplasmic proteins represent 30–35% of total muscle protein, they are water soluble and play a minor role in meat protein functionality; although myoglobin and other water-soluble compounds are responsible for meat color as the amount of myoglobin varies within species, age, and muscle fiber distribution. Dark muscles in turkey thigh are mainly comprised of red fibers and contain more myoglobin (0.6–2 mg/g) than light breast muscles (0.1–0.4 mg/g) as data reported by Froning and Mckee (2001). Lastly, stroma proteins are rather insoluble, comprise about 3–6% of total protein; collagen is the main component of this fraction in combination with small quantities of reticulin and elastin; but the amount of collagen and the age-associated collagen cross-linking influences meat texture (Miller 1994; Hettiarachchy and Gnanasambandam 2000).

Several factors influence poultry meat quality; for instance, post mortem condition at the time of removal from skeletal frame alters texture and meat quality. It is common that poultry carcasses are stored either under refrigeration or in ice for up to 8 h to ensure depletion of muscle ATP to prevent toughening (Lyon and Lyon, 2002; Sams 2001; Young and others 2004). After dead, individual cells remain alive and continue their metabolism; with the loss of blood and oxygen supply, cells gradually shift from aerobic to anaerobic glucolysis with production of lactic acid that accumulates in the muscle cells causing pH to decrease from neutrality to an acidic pH of about 5.7. The pH reduction alter the activity or some ATP producing enzymes until ATP depletion and the onset of rigor mortis. ATP is the primary energy supply that regulates the muscle contraction–relaxation cycle; when ATP concentration falls below 1 μM/g, muscle adopts a steady contraction state called rigor mortis as ATP is needed to break down the actomyosin complex between the overlapped thin and thick muscle filaments (Sams 2001; Ponce-Alquicira 2006a). The rate of pH decrease and the final pH value have a great influence on meat quality. A rapid drop in ATP and pH causes extensive drip loss because of muscle protein denaturation by combination low pH and high temperature exposition; this condition is known as PSE (pale, soft, and exudative) and it is associated to stress during slaughtering. In contrast, DFD-meat (dark, firm, and dry) has a high pH and a low level of lactic acid post mortem, this condition is caused by low levels of muscle glycogen when animals are exposed to a long-term preslaughter stress. Pale broiler breast fillets had similar marinade uptake but greater cooked losses than normal fillets (Cavitt and Owens 2001). Therefore, the myofibrillar protein and the physicochemical condition (PSE or DFD) mainly determine the functional properties and quality of raw materials.

Poultry marketing is moving towards cut-up parts and further processes commodities as shown in Figure 79.2. This change in the consumer preferences has shortened the time between slaughtering and cut-up or deboning. However, muscle cutting and deboning before rigor mortis will cause severe contraction or shortening, since the skeleton no longer restrains muscle length. Some processing plants use electrical stimulation to accelerate ATP depletion; this process involves application of an electric current either during bleeding or prior evisceration to accelerate ATP depletion, reducing the time for further processing and improves marinade absorption (Young and others 2004; Ponce-Alquicira 2006a). The main objective of marinating is to improve juiciness, flavor, and tenderness; these qualities are directly associated to protein hydration and water holding capacity. These functional properties are based into protein–water interactions that are affected by several intrinsic and extrinsic factors, therefore it is necessary to review those factors to ensure quality of marinated poultry products.

79.2.1 Water Holding Capacity

Water holding capacity (WHC) is the ability of meat to retain or absorb added water in the presence of an external force. Meat contains about 76% water; however, less than 5% is tightly bound to the hydrophilic sites while the additional 95% is hold by capillary forces between the thick and thin filaments, or physically entrapped within muscle structure. WHC is largely influenced by the contractile miofibrillar proteins organization, based in the distribution of hydrophobic and hydrophilic groups as well as in the charge and molecular flexibility of muscle proteins. All these characteristics may vary according to the post mortem state. WHC is elevated immediately after slaughter, but after the onset of rigor mortis, meat becomes rigid and WHC starts to decrease. After some time, endogenous muscle proteases cause morphological changes that disrupt the cytoskeleton organization. As a consequence, meat becomes soft and tender again with a slight rise in pH and WHC during post rigor. However, WHC is also affected by extrinsic factors such as pH and temperature, meat integrity, salt concentration, and addition of other nonmeat components (Lawrie 1998).

Myofibrillar proteins have the lowest water affinity at a pH set in the isoelectric point (pI) at pH $\sim$ 5.1, as they have a cero net charge and tend to aggregate; however, proteins become more negatively charged and repulsive forces between them increase at pH values close to neutrality, allowing more water to interact with the hydrophilic protein sites, thus increasing WHC. Changes that take place post mortem alter WHC as pH decreases and myofibrillar proteins are tightly bound forming the actomiosin complex. Addition of NaCl up to 0.6 M (2–3.5%) reduces electrostatic interactions between proteins, increases protein extractability, solubility and water binding in both breast and thigh muscle. Additionally, alkaline phosphates in combination with salt increases pH, myofibrillar protein solubilization and WHC (Zheng and others 2000; Li and others 2001; Aktas and others 2003). Furthermore, mechanical work during tumbling and massaging disrupts the muscle fibers facilitating water absorption and swelling; but an excess of mechanical work can lead to muscle fibers disintegration and protein denaturation due to an increase in temperature and excessive shearing; those denatured proteins as in PSE muscle form aggregates that have low water affinity (Lawrie 1998; Totosaus-Sanchez and Guerrero-Legarreta 2006).

79.2.2 Texture

Texture as well as color and flavor influences consumer's acceptance and poultry meat quality. This attribute is affected by the amount of collagen and the age-associated collagen crosslinking degree; therefore, meat from older animals is likely to be tougher than meat from young animals. In addition, meat from muscles involved in locomotion is higher in connective tissue than meat obtained from muscles used for structural support; for instance, Wattanachant and others (2004) reported that total collagen contents of broiler *pectoralis* and *biceps femoris* muscles were 3.86 mg/g and 8.70 mg/g, respectively.

On the other hand, the final pH and myofibril organization play an important role for meat texture; the biochemical and physical conditions during rigor affects the final contractile state and thus meat texture. The Z-line structure and the sarcomere length, that is, the distance between two adjacent Z-lines is primarily responsible of meat tenderness. As a result of rigor mortis, the sarcomere length decreases and muscle becomes tough;

later during post rigor, morphological changes affect the cytoskeleton in which Z-lines get disorganized, and meat becomes soft and tender again. The calcium activated sarcoplasmic factor (CASF) or calpains are proteases with an optimal activity at pH around 7, primarily responsible for degradation of desmin and conectin present in the Z-line, as well as M-line proteins, troponin T, and tropomyosin. Furthermore, the lysosomal proteases called cathepsins alter troponin T, myosin and actin in a synergistic action (Miller 1994; Pérez-Chabela and others 2005). To achieve optimum texture, poultry processing involves chilling and aging for 4–8 h after evisceration to allow rigor development and post-rigor tenderization (Miller 1994; Zheng and others 2000). It has been reported that marination at either high or low pH had positive effects in texture and WHC. Additionally, cooking losses were decreased as a result of the loss of M-line at low pH, and due to the loss of Z-line when marinating at high pH. The use of organic acids such as 0.1 M acetic or lactic acids, or red wine marinade induces a rapid degradation of myosin heavy chains, titin and nebulin, probably caused by activation of chatepsins as compared with nonmarinated meats (Oreskovich and others 1992; Hwang and others 2000; Lin and others 2000).

79.2.3 Color

Meat color of either raw or cooked varies according to species, muscle function, age, storage conditions, and further processing. Myoglobin is the mayor pigment in meat but its content varies according to the muscle physiological role. For example, high use leg muscles have a higher myoglobin content as they need it to store and deliver oxygen to support ATP production during long-lasting contractions. In addition, the myoglobin content increases with age, meat from older animals is darker than the one obtained from younger animals; also male birds have higher myoglobin than females (Claus an others 1994; Miller 1994; Wattanachant and others 2004; Pérez-Álvarez 2006). The mioglobin content is three times higher in turkey dark meat (0.37 mg/g) than in white meat (0.12 mg/g) obtained from 14 weeks aged male birds; but turkey white meat obtained from 24 weeks male birds has a mioglobin content of 0.37 mg/g. Hemoglobin is the mayor pigment in blood and also contributes to meat color; the hemoglobin content in meat varies according to conditions prior to and during exangination, which influence blood removal. Improper bleeding practices result in a great amount of haemoglobin within muscles. Other meat pigments such as cytochromes, catalase, and flavins have a minor influence in color (Miller 1994; Pérez-Álvarez 2006).

Deleterious changes during storage and further-processing have a less dramatic impact in poultry meat color than that for red meats. The bloody, undercooked appearance or "pinking" is one of the most common color problem in cooked poultry meat and it is associated to the presence of blood. However, several factors such as bird age, diet (intake of nitrites or moldy feed animals); preslaughter transport and handling, improper stunning; as well as meat aging, processing, and cooking method, among others are involved in meat "pinking." It has been suggested that the ferrous *heme* iron from metmyoglobin can weakly bind to other nitrogen-containing compounds such as denaturated proteins or nicotinamide causing "pinking"; but a decrease in the oxidation-reduction potential and CO-myoglobin formation are also associated this color phenomena (Claus and others 1994; Nam and others 2001; Smith and Northcutt 2004).

Other color defect is called bone darkening found in cooked chicken meat, where the tissue around the bone is discolored with a black appearance, this color defect is commonly observed when poultry is frozen prior to cooking, as a result of bone marrow

leaking trough the bone onto surrounding meat, which becomes dark during cooking; blast freezing and reheating increase darkness in raw and cooked bone-in broiler tights, but removal of femur prior to freezing decreased this problem. The less common color problem that affects poultry is the intense red, bloody discoloration of bone-in fully cooked products; it seems that the presence of blood derived from hemorrhages and bruises is more important than bone marrow for the dark and red discoloration of broiler meat. However, marinating with phosphates citric acid and EDTA may reduce the redness discoloration in both raw and cooked chicken breast meat (Smith and others 2001; Smith and Northcutt 2004).

79.2.4 Flavor

Poultry flavor is composed by meat-like water soluble reducing sugars, amino acids, and lipids. Free sugars, free amino acids, peptides, nucleotides, and other nitrogenous components such as thiamine, are among the main water-soluble flavor precursors, although frozen storage and cooking may change their concentration. Raw poultry (as other meats) has a light metallic bloody flavor, but during heating and storage, nonenzymatic browning reaction, Strecker degradation, lipid oxidation, thiamine degradation, and proteolysis among other chemical reactions, take place to create the characteristic poultry flavor (Farmer 1999; Ang 2000). Undesirable tastes may derive from low molecular weight compounds, lipid oxidation, or protein degradation that happen during long-term storage, or by cell disruption and releases of haemoglobin and lipids from the bone marrow (Miller 1994; Froning and Mckee 2001; Smith and Acton 2001; Ponce-Alquicira 2006b,c). It is well known that marinating improves flavor by incorporation of salt, phosphates, sugars, and several seasonings but care must be taken as formulation errors can yield to flavor deterioration and product rejection. Salt may promote lipid oxidation and off-flavor development; whereas, an excess in of phosphates will cause bitterness, therefore, correct formulation, high quality materials, and good manufacturing practices will ensure the flavor attributes expected by consumers.

79.3 FUNCTIONAL INGREDIENTS

Ingredient formulation is critical in new product development to maximize quality and yield, attending both producer and consumer requirements. Incorporation of the appropriate functional ingredients at effective levels is crucial for a successful marinating process. Salt, phosphates, water, seasonings, sweeteners, polysaccharides, vegetable or dairy proteins, acids, antimicrobials, and antioxidants, among other additives are common ingredients present in the marinade. These components add value to poultry meat as they increase WHC, improve yield, juiciness, flavor, tenderness, extend product storage-shelf, and control costs (Bacon 2001; Keeton 2001). However, processors have to keep in mind that food additives are minor constituents intentionally added to enhance food quality and strict regulations are imposed to control their use.

Marinade formulation needs to be created also according to the postprocessing handling; for instance, frozen cooked products that will be cooked twice (during processing and before eating) must retain even more moisture, therefore ingredient technology needs to contemplate this actions (Bacon 2001). Tables 79.1 and 79.2 present the typical formulation of marinade for chicken fajita strips.

TABLE 79.1 Marinating Solution Formulation for Chicken Fajita Strips.[a]

	Percent of Total
Water (maximum)	65.63
Spices/seasonings (maximum)	25.00
Binders[b]	6.25
Sodium phosphates (maximum)	3.12
Total	100.00

[a]The marinating solution percentage incorporated within tumbled or massaged poultry meat must not exceed 16% over the original ready-to-cook weight.
[b]Binders include starch, modified starch and/or isolated soy protein.
Source: Commodity Specification. Agricultural Marketing Service, USDA. June 2005.

79.3.1 Salt

Sodium chloride or common salt is considered safe by the U.S. Food and Drug Administration (FDA) and receive the status of GRAS (generally recognized as safe). Salt enhances flavor and taste, increases ionic strength, protein solubility, and WHC, also reduces shrinkage and cooking loss, limits drip loss following freeze-thaw, and acts in combination with sodium nitrite to prevent outgrowth of *Clostridium botulinum*. Salt may be considered as antibacterial because, at high concentration (above 5%), it restricts bacterial growth by lowering the water activity or free water molecules in foods; therefore, it is used to improve flavor, texture, and as a basic curing agent for meat preservation. However, the recommended salt concentration in finished poultry products varies from 1.5% up to 2%. Salt levels in meat products are decreasing due to consumer healthy concerns; thus, some products may be low in sodium by partial substitution of sodium by potassium, calcium, or magnesium salts. However, marinate absorption is related to the salt type and the ionic strength needed to dissociate proteins and increase WHC. Samples marinated with calcium salts retain less water than those marinated with sodium chloride, thus low sodium marinades should be formulated in a way to achieve the appropriately muscle functionality and yield. Moreover, care should

TABLE 79.2 Proportion of Spices and Seasonings for Chicken Fajita Strips Marinade.

	Maximum Percent
Salt	48
Sugar[a]	18
Sodium phosphates	16
Black pepper	10
Onion powder	10
Garlic powder	10
Chilli powder	5
Binders (food starch)	5
Grilled and/or smoke flavoring	5
Citric acid/lemon juice/lime juice/vinegar	5
Red pepper	2
Other spices, extract, or ingredients	20
Total	100

[a]Sugar may be sucrose, brown sugar, dextrose, or a combination thereof.
Source: Commodity Specification. Agricultural Marketing Service, USDA. June 2005.

be taken as salt may contain metal contaminants that accelerate lipid oxidation and rancidity diminishing flavor qualities (Aktas and others 2003; Brant 2003).

79.3.2 Phosphates

Marinade solutions containing phosphates in combination with salt enhance WHC, overall moisture, juiciness, and yield; while reducing cooking and purge loss. Phosphates also limit drip loss following freezing-thaw and increase tenderness. Muscle proteins show the lowest water binding capacity at a pH close to its pI (pH $\sim$ 5.4). After slaughtering, meat pH approaches to the isoelectric point and WHC decreases, but phosphate addition increases meat pH to an optimal range of 6–6.4. The USDA establishes a maximum phosphate content of 0.5% in the finished product, but levels close to the maximum limit may cause soapy and bitter taste, and may also produce a rubbery like texture; in consequence, most formulations include 0.3–0.4% phosphates in the final product (expressed as phosphate pentoxide, P_2O_5); in addition, only clear solutions may be injected into poultry (Li and others 2000, 2001).

There are several chemical forms of phosphates, but not all produce the same effect; thus, choices can be made according to the required functionality (see Table 79.3). Alkaline phosphates increase meat pH and ionic strength, also they break down crosslinks between myofibrillar proteins and increase the number of hydrophilic protein groups available for water binding, thus increasing WHC, juiciness, and product yield during thawing, reheating, and storage (Li and others 2000, 2001). Phosphates also help to maintain color, enhance meat flavor, and delay oxidative rancidity by chelating metal ions. Phosphates vary

TABLE 79.3 Properties of Some Food Phosphates Used in Meat Industry.

Name	Acronym	Formula	Solubility[a]	pH[b]
Ortophosphates				
Monosodium dihydrogen phosphate	MSP	NaH_2PO_4	48	4.6
Disodium monohydrogen phosphate	DSPA	Na_2HPO_4	11	9.2
Trisodium orthophosphate	TSPA	Na_3PO_4	13	11.8
Monocalcium phosphate	MCP	$Ca(H_2PO_4)_2-H_2O$	Slight	4.6
Pyrophosphates				
Tertrapotassium pyrophosphate	TKPP	$K_2P_2O_7$	>60	10.3
Acid sodium pyrophosphate	SAPP	$Na_2H_2P_2O_7$	13	4.2
Tetrasodium pyrophosphate	TSPP	$Na_4P_2O_7$	6	10.2
Tripolyphosphate				
Sodium triphosphate	STP	$Na_5P_3O_{10}$	13	9.8
Trisodium dipotassium triphosphate	SKTP	$Na_3K_2P_3O_{10}$	37	10
Polyphosphates				
Sodium hexametaphosphate (phosphate glass)	SHMP	$Na_{15}P_{13}O_{40}-Na_{20}P_{18}O_{55}$	>60	6.9
Sodium trimetaphosphate	STMP	$(NaPO_3)_3$	23	6.7

[a]solubility expressed in g/100 g, 25°C.
[b]pH of 1% solution.
Source: Rocha de McGuirre 2003; http://www.sigma-aldrich.com.mx, February 2006.

in their solubility and pH, therefore, several types of phosphates alone or in a combination thereof are commonly used in meat processing (see Table 79.3). These include ortophosphates, diphosphates, or pyrophosphates (PP), tripolyphosphates (TPP), polyphosphates, and tripolyphosphates or "glass" phosphates, and metaphosphates (MP). Once incorporated into meat, some phosphates may be hydrolyzed nonenzymatically or enzymatically by muscle phosphatases to monophosphate increasing muscle pH at various rates according to their chemical structure. Diphosphates are the most effective chemical form for marinating but have relatively low solubility; thus, diphosphates are normally combined with more soluble, longer chain phosphates such as tripolyphosphates and hexametaphosphates in a 10/90 ratio. Whereas, orthophosphates have less impact on water binding for marinated poultry products. Therefore, the correct choice of phosphate blend may improve quality and reduce cost (Li and others 2000, 2001; Zheng and others 2000; Rocha 2003).

79.3.3 Species and Flavorings

In general poultry meat posses a delicate flavor and adapts very well to any flavoring combination according to the consumer taste preferences and flavor profile, either traditional or emerging tastes (Ponce-Alquicira 2006b). There is an innumerable source of flavorings that could be used for poultry marinating to impart or modify flavor, such as spices, chilli peppers, oils, vinegars (apple, rice, red wine, etc.), fruit juices or nectars, or synthetic flavors (see Table 79.4). Spices are marketed ungrounded or as coarsely or finely ground powders, but after grinding aroma is lost gradually during storage; in addition powders are commonly contaminated with microorganisms and may accelerate food spoilage. Therefore spice extracts, essential oils, oleoresins, or microencapsulated flavorings are commonly added in industrial scale food preparation, since they are free of microorganism, uniform, and easy to handle. The way in which these ingredients are added into the marinade will depend on the marinating process, in particular it is preferable to use flavor concentrates and/or oleoresins when marinating by injection to avoid needle blocking caused by the presence of small particles. In addition to spices, smoke flavor has been used for years to enhance and modify flavor for barbecue and grilled marinated products. Smoke components include phenols, acids, and carbonyl compounds derived from the pyrolysis of lignin and cellulose. Among them, syringol is a phenolic compound derived from lignin, and the main responsible for the smoke and hickory flavor; while carbonyl compounds have minor flavor impact (Rocha 2000; Brant 2003; Ponce-Alquicira 2006b,c).

The flavoring industry is aware of the potential that spices and seasonings have on product development to create healthy and enjoyable dishes, and to reinvent classic tastes, throughout the correct selection of spices and flavorings. Nowadays, species are seen as functional ingredients that not only improve flavor, but enhance salty perception, alleviate diabetes and high cholesterol, promote antioxidant, and antimicrobial activities, and also help to expand and reinvent conventional cuisines. As a result of globalization and immigration, consumers are more familiar to a wide range of cultures, and willing to taste other cuisines such as African, Asian, Brazilian, Indian, Italian, Mexican, Mediterranean, Cajun, Buffalo style, and so on. Thus, flavorings can also help to reshape or create foods such as the great variety of marinated poultry products. The Mexican cousin flavor can be obtained by marinating with chipotle, jalapeño or habanero chili-peppers; fresh coriander, paprika, as well as *mole* (sauce made of chocolate, ancho chili pepper, tomato, garlic, onion, nuts, and spices), *adobo*

TABLE 79.4 Spices Used in Meat Industry.

Group	Common Name	Latin Name	Flavor Characteristics
Leaves	Basil	*Ocimum basilicum*	Hay-like and minty, slightly bitter and musty
	Chervil	*Anthriscus cerefolium*	Sweet, aromatic
	Chives	*Allium schoenoprasum*	Delicate, peppery-onion
	Dill	*Anethum graveolens*	Sweet, green/grassy, tea-like
	Marjoram	*Origanium hortensis*	Minty, aromatic and slightly bitter
	Mint	*Mentha spicata L. (spearmint) Mentha piperita L. (peppermint)*	Aromatic, strong, sweet flavor with a cool aftertaste
	Oregano	*Origanum spp. or Lippia spp. (Mexican)*	Strongly, camphoraceous aroma, slightly bitter, pungent
	Parsley	*Petroselinum crispum*	Green and vegetative
	Rosemary	*Rosmarinus officinalis*	Pine-woody aroma with fresh, bittersweet
	Sage	*Salvia officinalis*	Medicinal, piney-woody
	Savory	*Satureja hortensis*	Aromatic, piquant and a fragrant aroma
	Tarragon	*Artemisia dracunculus*	Aromatic, licorice-like
	Thyme	*Thymus vulgaris*	Minty-green, hay-like, and musty notes.
Fruits	Allspice	*Pimenta dioica*	Pleasant blend of cinnamon and clove
	Anise	*Pimpinella anisum*	Pleasant, licorish-like, similar to fennel
	Caranway seed	*Carum carvi*	Pleasantly aromatic and biting
	Chilli pepper	*Capsicum annum*	Sweet to moderate heat, and biting notes
	Coriander	*Coriandum sativum*	Mildly sweet and spicy
	Pepper	*Capsicum frutescens*	Tobacco, hay-like, hot/pungent
Seeds	Cardamon	*Elettaria cardamomum*	Soapy with menthol notes
	Cumin	*Cuminum cyminum*	Distinctive, slightly bitter
	Mustard	*Brassica hirta Brassica juncea*	Fresh aroma and a pungent, slightly biting
	Nutmeg	*Myristica fragans*	Strongly aromatic, citrus, piney
	Sesame	*Sesamum indicum*	Nut-like
Rhizomes	Ginger	*Zingiber officinale*	Lemon/citrus, soapy and musty/earthy notes
Flowers	Cloves	*Syzygium aromaticum*	Strong, pungent, sweet and hot
Barks	Cinnamon	*Cinnamomun zeyland, Cinnamomun cassia*	Sweet and pungent
Color spices	Annatto seeds	*Bixa orellana*	Rich yellow/orange color, musky-flavour
	Curcuma	*Curcuma longa*	Yellow like color; aromatic and spicy fragrance
	Papika (sweet red pepper)	*Capsicum annum*,	Bright Red color; sweet in flavor with fragrant aroma
	Saffron	*Crocus sativus*	Rich yellow, strong perfume and bitter honey-like taste

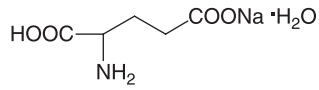
Source: Belitz and Grosch 1999; http://www.mccormick.com/(February 2006); http://www.culinarycafe.com/Spices_Herbs/ (February 2006).

Figure 79.3 *Variety of fresh and frozen marinated poultry meat products (chicken and turkey parts with herbs, adobo, achiote, butter, oriental, arrachera, natural, smoke, mesquite, teriyaki, and California marinade flavors).*

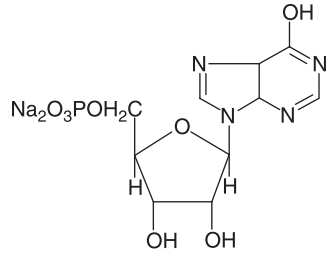
(spiced red chili sauce), or *achiote* (Yucatan-style sauce from ground annatto seeds, onion, garlic, and tomatoes), among others. Caribbean flavors include cinnamon, pineapple, coconut, orange, and lemon juices. The Asian marinade includes garlic, ginger, coconut, lemongrass (*Cymbopogon citratos)*, mushrooms, sesame seeds, soy sauce, and teriyaki sauce (sugar, soy sauce, and sake marinade); while the Hindu influenced marinade may include strong flavors from cardamom, ginger, curcuma, cumin, and hot chilli-peppers). These days, marketing for marinade products in the United States are reoriented to the "Nuevo Latino flavor trend" with flavor profiles such as citrus, chilli peppers, heat, and fresh herbs. Therefore, flavorings have gone to a more complex mixtures as consumers will look at the wide variety; upcoming products include a combination of Asian and Caribbean flavors prepared with ginger, honey, orange, garlic, and pineapple; in addition to, flavor blends such as fruity-sweet combined with heat, or cool and spicy flavor notes (Rocha 2000, 2001; Brandt 2003; Pszczola 2005; http://www.mccormick.com 2006). In general, poultry meat adapts very well to all sorts of flavor trends, and this is the cause for the large increase in consumption for further processed poultry items over other meats such as those presented in Figure 79.3.

79.3.4 Flavor Enhancers

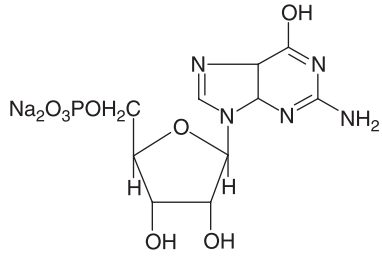
There are several compounds that enhance the original taste or flavor; the most widely used flavor enhancers are monosodium glutamate (MSG), inosine-5′-monophosphate (IMP) and guanosine-5-monophospahte (Fig. 79.4), and hydrolyzed vegetable protein (HVP). They are currently accepted as GRAS food additives, but enhancing mechanisms are not well understood. It has been suggested that these compounds are responsible for the *umami* flavor perception, they act in a synergistic mode to increase salty, and sweet flavors, due to an increase in the time of residence of active flavorings with the gustative receptors. The amount of MSG added varies from 0.1% to 1%, whereas, levels for IMP



monosodium glutamate



disodium inosine-5´-monophosphate



sodium guanosine-5´-monophosphate

Figure 79.4 *Chemical structure of common food flavor enhancers. (Adapted from Belitz and Grosch 1999; Hettiarachchy and Kalapathy 2000.)*

and GMP range from 0.002% to 0.03% (Hettiarachchy and Kalapathy 2000; Marcus 2005; Ponce-Alquicira 2006c).

79.3.5 Sweeteners

Dextrose, sucrose, corn syrup solids, sorbitol, manitol, honey. Molasses and other sweeteners are commonly added to marinated poultry products in order to increase moisture retention, and enhance flavor by reducing salt harshness. These compounds differ in sweetness and taste intensity; although, temperature, pH, and the presence of other sweet compounds could modify also their taste perception. Sugars participate in Maillard and caramelization browning reactions generating color and flavor compounds (Ponce-Alquicira 2006c). Therefore, the use of sweeteners is somewhat self-limiting, because they can cause excessive browning and sweetness. Maltodextrins and corn syrup solids are tasteless and water soluble products, which offer reduced sweetness and browning, while providing solids and a slight increase in viscosity according to their molecular weight and on their dextrose equivalents (DE), thus helping to retain the marinade in the poultry product (Keeton 2001; Brant 2003). Furthermore, honey is high in fructose, not only contributes to retain water and flavor development; but honey also reduces lipid oxidation improving quality as reported by Hashim and others (1999); mainly because honey contains various phenolic compounds such as α-tocopherol,

ascorbic acid, β-carotene, catalase, and peroxide, which can function as antioxidants. Besides, it has been claimed that honey inhibits formation of heterociclic aromatic amines during cooking and grilling, thus resulting in a reduction of mutagenicity in cured poultry (Shin and Ustunol 2004).

79.3.6 Binders and Extenders

Water-binding agents such as starches, hydrocolloids, and nonmeat proteins are used alone or more often in combination to help minimize marinade purge. These ingredients enhance the yield and quality of marinated poultry products as they retain moisture, provide freeze-thaw stability, and reduce losses during cooking. Nonmeat proteins used in poultry meat products include soy flour, soy protein concentrate and isolate, nonfat dry milk, dry whey, whey protein concentrates and isolates, and plasma proteins. These proteins prevent shrinkage during cooking and retain juiciness, contribute to nutrition and flavor, and they have a significant effect on the pH of meat systems because of their buffering capacity. On the other hand, polysaccharides include starches and hydrocolloids. Native and food grade modified starches and cellulose derivates are widely used due to their low cost and availability; they bind four times their weight in moisture, and contribute to texture. Levels vary from 1 to 3.5 and up to 18% according to the application and regulatory restrictions. Hydrocolloids such as carrageenan, konjac, and alginate, among others are long-chain polymers which dissolve readily in water to give a thickening effect at levels below 2%. Label restrictions sometimes limit the amount of total binders included; once a limit has been established, the best combination of synergistic ingredients should be used to give the desired moisture and texture for the product; that can become a very complex matrix of ingredients to achieve the desired product effect (Keeton 2001; Brant 2003; Valdés Martínez 2006).

79.3.7 Acidulants

The most common acidulants are acetic, citric, fumaric, lactic, and phosphoric acids or their salts, as well as, glucono-delta-lactone (GDL), which produce acid during hydrolysis. Acetic acid is used in foods as vinegar; it is produced by *Acetomonas* and *Acetobacter* bacteria through fermentation from grapes, grains, cane sugar, apple or pineapple juice, and other substrates. Each type of vinegar has its own distinctive flavor profile such as acid, fruity, musty, and so on Vinegar addition reduces pH, controls the microbial growth, and enhances flavors. Lactic acid and sodium lactate are widely used in meat processing to reduce water activity and inhibit spoilage bacteria. Citric acid is widely distributed in nature in both vegetal and animals tissues. It is used for flavor enhancement because its ability to chelate potentially prooxidative metal ions, thus retarding oxidation and increasing effectiveness of antioxidants. In meats, citric acid is used at levels below 0.01% to prevent oxidative rancidity protecting flavor and color. GDL slowly hydrolyzes releasing acid; it is commonly used in cured poultry products to reduce the cure time, to inhibit undesirable microorganisms, to promote color development, and to reduce nitrate and nitrite levels (Dziezak 1990; Belitz and Grosh 1999).

Latin, Asian, and Caribbean cuisines incorporate acids and citrus flavors (lemon, lime, and orange), that are difficult to apply in meats because highly acidic marinates ($pH < 5$) may accelerate protein denaturation and proteolysis, causing meat to become too soft in detriment of texture. For instance, Hwang and others (2000) claimed that marinated

goose breast with 0.1 M lactic or acetic acids for 24 h at 5°C exhibit degradation of myosin heavy chain, titin, and nebulin myofibrillar proteins; thus the marinade formula must balance the acidic effect to obtain the appropriate texture (Brant 2003).

79.3.8 Antioxidants

Lipid oxidation is one of the main problems for poultry meat due to disruption of the integrity of muscle membranes during processing, and freeze-thawing. Further reactions involved in lipid oxidation leads to "warmed-over flavor" (WOF) development during cooking. The term WOF refers to the rapid development of off-flavor in cooked meat during refrigerated storage, and it has become a serious problem for precooked and reheated poultry, as well as in fresh ground and restructured meat; along with cooked, refrigerated, or irradiated poultry products. Lipid oxidation may be initiated by catalysis of heme iron present in haemoglobin and myoglobin, and also by enzymic lipid peroxidation, in conjunction with higher polyunsaturated fatty acids of membrane phospholipids (Ahn and others 1993; Hettiarachchy and Gnanasambandam 2000; Armitage and others 2002; Lee and Ahn 2003; Sucan 2004; Rababah and others 2005; Ponce-Alquicira 2006b,c).

Several substances have been examined to prevent lipid oxidation in meat products, antioxidants such as butyl hydroxytoluene (BHT) and butyl hydroxyanisole (BHA), tertiary-butylhidroquinone (TBHQ), proppyl gallate, and α-tocopherols are free radical scavengers that react with peroxyl or alcoxyl radicals and terminate the chain reaction of peroxidation (Fig. 79.5). All this antioxidants in addition to metal chelating agents (EDTA, citric acid, and phosphates) are commonly used to reduce lipid oxidation in both raw and cooked poultry meat (Anh and others 1993; Hettiarachchy and Gnanasambandam 2000). However, since BHA has been identified as a carcinogen in laboratory animals, there is an increasing interest to study other natural antioxidants. Plant extracts

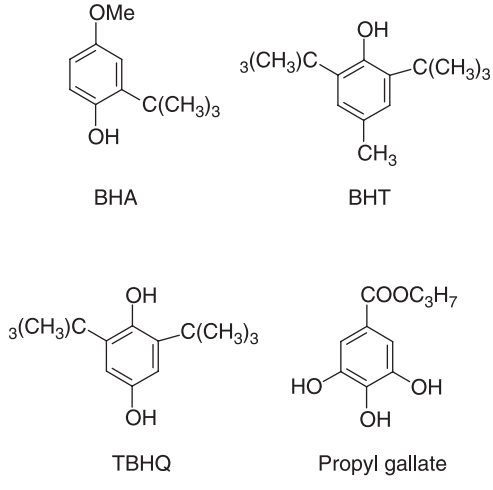


Figure 79.5 *Chemical structure of butyl hydroxyanisol (BHA), butyl hydroxytoluene (BHT), teritiary-butyl hydroquinone (TBHQ), and propyl gallate (PG). (Adapted from Belitz and Grosch 1999; Hettiarachchy and Kalapathy 2000.)*

such as green tea, grapefruit seed, cocoa leaves, oregano, and rosemary extracts; as well as, tea catechins, vitamin E, and honey could retard lipid oxidation (Tang and others 2001; Armitage and others 2002; McKibben and Engesth 2002; Basmacioglu and others 2004; Hassan and Fan 2005; Rababah and others 2005; Hernández-Hernández and others 2006; Peña-Ramos and others 2006). However, results in terms of oxidative prevention are unequal and usually modest with large variations, according to storage conditions, aerobic or anaerobic packaging, amount of free polyunsaturated fatty acids, and integrity of raw tissues (Lee and Ahn 2003; Lee and others 2003; Mielnik and others 2003; Soares and others 2004).

79.3.9 Antimicrobials

The use of antimicrobials is intended to reduce the number of microorganisms and subsequent spoilage. The three most common preservatives are sorbates, propionates, and benzoates. In general, sorbates are the more effective preservatives against a wider spectrum of food spoilage microorganisms than benzoates or propionates for mildly acidic foods (pH

TABLE 79.5 Antimicrobials in RTE Meat and Poultry Products.

Name	Amount	Reference	Labeling Requirements
Aqueous octanoic acid (0.1–1.0%) and glycerin or polysorbate (0.5–5%), pH 1.5–4	Applied as pray 0.12 mL/ in^2 of product surface	Acceptability determination	Not under the accepted conditions of use
Acidified sodium chloride	500–1200 ppm in combination with any GRAS acid to achive a pH 2.3–2.9	21 CFR 173.325	Not under the accepted conditions of use
Cetylpyridinium chloride	Applied as fine mist spray (1% aqueous solution)	21 CFR 173.325	Not under the accepted conditions of use
Egg white lysozyme	2.5 mg per pound finished product	GRAS notice No. 000064	Listed by common name
Blend of citric acid, hydrochloric acid, and phosphoric acid	Sufficient for purpose	Acceptability determination	Listed by common name
Hops beta acids	2 mg per pound on cooked meat and poultry	GRAS notice No. 000063	Listed by common name
Lauramide arinine ethyl ester (LAE)	Applied to the surface not exceed 200 ppm	GRAS notice No. 000164	Listed by common name
Nisin	2.5 mg per pound on cooked poultry	GRAS notice No. 000065	Listed by common name
Sodium citrate buffered with citric acid pH 5.6	$<$1.3% product formulation	Acceptability determination	Listed by common name
Sodium metasilicate	$<$2% by weight of marinade	Acceptability determination	Listed by common name

Source: Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products FSIS Directive 7120.17120.1 Amend 6. Available at http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1 Amend 6.pdf (03/2006).

5.5–6.0) as most poultry products. Other common antimicrobials include acidified sodium chloride, organic acids, peptides, trisodium phosphate, bacteriocins, natamycin, cetylpyridinium, lactoferrin, lysozyme, lactoperoxidase, and so on. However, their use in poultry processing requires approval and need to complaint with regulatory limits, according with the type of products. Recent applications include combination of antimicrobials for synergistic effect with packaging and antimicrobial components such as grape seed, rosemary, and garlic natural extracts, as well as honey, among others (Arrit and others 2002; Pszczola 2002; Ricke 2003; Joerger 2003; Oyarzabal 2005). Antimicrobials are listed in the table of safe and suitable ingredients for use in the production of meat and poultry products under FSIS Directive 7120.1 regulations that are presented in Table 79.5.

79.4 MARINATING SYSTEMS

Soaking is the simplest method for marinating, where poultry is placed into a container with the marinade and kept for about 24 h under refrigeration below 40°F (4.4°C). This process is low in cost and offers good adhesion of skin-on products; however, inconsistent marinade uptake, extra labor, and cooler space requirements are some disadvantages associated to this marinating process. Therefore, most poultry products are typically injected with marinade and the subjected to mechanical forces to enhance the action of salt and phosphates. Injection traps the marinade within the meat fibers and promotes even distribution throughout the entire poultry item to impart the desired effect. The marinade is pumped in and injected using single-needle injectors for smaller pilot-plants, or using large multineedle injectors, according to the type and cut being treated. Injectors can be adjusted for specific fluid pickup, as well as pressure and rate. Once meat is released from the injector, it may be cooked, tumbled, or packaged, depending on the desired finished product. Tumblers or tumbler-massagers are equipped with baffles or paddles to maximize physical contact and provide mechanical agitation between meat and the marinade solution. The meat and the marinade solution or dry mix are placed into a revolving container and allowed to tumble, ideally at 33–35°F (0.5–2°C); vacuum, rotation speed, total time, and temperature conditions are set according to the product allowing a rapid marinade uptake. Mechanical action helps to break the sarcolema and endomysium, allowing better brine penetration and greater swelling of the myofibrils. Tumbler design varies by type of mechanical action, from gentle motion to a more violent, cement-mixer type. The length of mechanical agitation depends on the type of cut, and tumbler speed. Chicken breasts may tumble for 15–30 min with good marinade uptake (Li and others 2000, 2001; Smith and Acton 2001; Brant 2003).

Large pieces or whole poultry carcass may be subjected to tumbling or massaging after injection to retain more marinade, with less drip losses during cooking, but required less force to shear than noninjected poultry meat items. However, injected chicken had a glossier and moister appearance; more intense flavors and more tender texture than immersed meats (Hashim and others 1999).

Marinade pickup is restricted to an 8% in boneless poultry and 3% in bone-in poultry. Processing parameters that affect marinating pickup and retention include marinade temperature, solubility of marinade ingredients, adequate pressure, and residence time for needle injectors; or appropriate mechanical action, vacuum and time in the case of tumblers or massagers (Toledo 2001). The amount of marinade injection generally depends on the pumping pressure, but injection is inadequate for marinades containing

large solid particles due to needle obstruction. Poultry cuts that have weak connective tissues are normally injected instead of tumbled because they will break apart in a tumbler. Moreover, blending is recommended for small pieces or ground meat while tumbling is preferred for a large variety of products such as skinless, skin-on, bone-in, whole muscles, and chopped pieces, but may not be appropriated for some fragile products or those with skin loosely attached (Smith and Acton 2001).

79.5 PACKAGING AND LABELING

Poultry marinated products are packaged in order to unitize the product according to the required marketing specifications. Packaging materials act as a barrier between the product and the environment, and must offer protection against microbial contamination, prevent moisture loss, physical and chemical deterioration. Plastic polymers are commonly used for primary poultry packaging due to their versatility, cost, and convenience. These polymers include polyethylene (PE), polypropylene (PP), ionomers, polyvinyl chloride (PVC), polyvinylidene chloride (PVdC), ethylene vinyl alcohol (EVOH), ethylene vinyl acetate (EVA), polystyrene (PS), polyamides (nylons), polyesters, polycarbonates (PC), and so on. Current practices for retail fresh poultry packaging include the use of high oxygen permeable polystyrene foam trays with a high oxygen permeable PVC or polymer-based stretch film over-wrap. Also poultry parts are placed in corrugated containers with plastic liners; the liner is gas-flushed with a modified atmosphere and sealed within the corrugated container. In addition EVA/PVdC/EVA or nylon/EVOH/inomer coextruded materials are commonly used for its oxygen barrier, toughness, heat resistance, and forming properties in modified atmosphere or vacuum systems. The leakage of food packages may cause microbial risk and increase chemical and autolytic spoilage (Randell and others 1995). Leakage is often caused by improper sealing or by mechanical damage during handling and transport. Emerging packaging technologies include edible coatings from polysaccharides, proteins and lipids; in addition to active packaging containing antimicrobials, antioxidants, moisture absorbers, oxygen scavengers, among others that increase hurdles to prevent deterioration (Gennadios 1997; Dawson 2001).

The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) shares responsibility with FDA for the safety of food additives used in meat, poultry, and egg products. The statutes and regulations require in all cases that ingredients must be listed on the product label, in the ingredients statement in order by weight, from the greatest amount to the least. Substances such as spices and spice extracts may be declared as "natural flavors," "flavors," or "natural flavoring" on meat and poultry labels without naming each one. This is because they are used primarily for their flavor contribution and not because of their nutritional contribution. On the other hand, substances such as dried meat, poultry stock, meat extracts, or hydrolyzed protein must be listed on the label by their common or usual name because their primary purpose is not flavor; they may be used as flavor enhancers, binders, or emulsifiers. They must be labeled using the species of origin, for example, dried beef, chicken stock, pork extract, or hydrolyzed wheat protein. Color additives must be declared by their common or usual names on labels and not collectively as colorings. In addition, date of packing is required in Title 9 of the Code of Federal Regulations (9 CFR), Section 381.126(a) and (b).

79.6 MICROBIAL CONSIDERATIONS

Poultry provides an excellent medium for the growth of spoilage and pathogen microorganisms; although *Salmonella* and *Campylobacter* spp. are the predominant foodborne pathogens associated to poultry and frequently implicated in human illness; even though, other pathogens are also present such as *Clostridium perfringens*, *Escherichia coli* 0157, *Listeria monocytogenes*, *Arcobacter*, and *Helicobacter* spp. Spoilage microorganisms include *Pseudomonas* spp., *Staphylococcus*, *Micrococcus*, *Acinetobacter*, and *Moraxella*. Microbial contamination of poultry takes place during the slaughtering and further processing procedures, by contact of the carcass with feathers, feet or intestinal contents that contain a high microbial load; also because of the use of contaminated equipment, and physical manipulation during deboning and further processing. Prevention of microbial contamination involves careful regulation and monitoring of the slaughtering and processing plants, proper handling, and storage at low temperature. Poultry as other meats is very perishable if stored under ambient conditions, but storage under low temperature such as refrigeration and freezing extends storage life. Several marinade ingredients have an antimicrobial activity such as salt, nitrates, phosphates, spices, sugars, and acids; in particular GDL, sodium, or potassium lactate, as well as sodium acetate, and diacetate can be added to poultry products to extend shelf-life, control pathogen growth, and improve flavor and moisture retention.

However, implementation of Hazard Analysis Critical Control (HACCP) and Quantitative Risk Assessment (QRA) systems in processing plants are essential to control contamination with spoilage microorganisms. The systems brings obvious benefits by optimizing plan hygiene, ensuring compliance with legislation, and providing evidence of processor outstanding conscientiousness, as microbiological safety and quality of poultry meat products are equally important to producers, retailers and consumers (Mead 2004).

79.7 CONCLUSION AND FUTURE TRENDS

Poultry marketing is moving towards cut-up parts and further processes commodities, therefore the poultry industry and researchers should build up advanced processing technologies to produce quality consumer-oriented products. Marinating is becoming widespread in the poultry industry in the United States, Latin America, and Europe, essentially because it improves not only sensorial qualities but also increases shelf-life and product yield during processing, defrosting and cooking. Marinating offers a great prospect to enhance poultry consumption and processing in terms of flavor adaptation to new and traditional flavor trends. Furthermore, marinated poultry can easily adapt to the emerging packaging technologies such as the active and edible coatings made from polysaccharides, proteins, and lipids; in conjunction with alternative food processing technologies like microwave radio frequency, ohmic and inductive heating, high pressure processing, or pulsed electric field, and other technologies under research.

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80

Poultry Sausages

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80.1 Introduction 775
80.2 Mechanically Deboned Poultry Meat 776
80.2.1 Nutritional Properties 776
80.2.2 Microbiological Properties 777
80.2.3 Functional Properties 777
80.3 Poultry Sausage Manufacture 778
80.3.1 Effect of Gelling Polysaccharides on MDPM Sausages Texture 779
References 780

80.1 INTRODUCTION

World poultry meat consumption is of significant economic importance in more than 50 countries worldwide. After pork, poultry is the second most consumed meat globally in the world meat market. There are three classifications for the factors driving the future demand for poultry: demographic, economic, and nutritional. First, the increase in demographic population, specifically in urban areas, requires an increase in animal protein production, in which poultry industry can satisfy. Second, the cost of feed grains, oil seeds, and other primary feed ingredients make poultry a relatively low animal-origin food compared to competing foods, such as pork and beef. Third, there is an increased poultry preference related to nutrition and health concerns. In addition to these factors, poultry product development, using bird parts not generally consumed can meets the market chain requirements and end-consumer desires (Roenigk 1999).

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The increase in poultry consumption provides considerable quantities of leftover parts including the back, neck, and frame meat available for mechanically deboning. The remaining adhering meat is recovered using mechanical separators, enabling an efficient use of whole chickens carcasses, which cannot be economically deboned by hand (Smith and Brekke 1985; Perlo and others 2003; Pettersen and others 2004). An alternative to employ this important animal protein source is the manufacture of sausages from mechanically deboned poultry meat.

80.2 MECHANICALLY DEBONED POULTRY MEAT

The process of mechanical deboning was created to make all of the meat left on the carcass, neck, and back of the chicken commercially viable, providing a new raw material for processed meat products, called mechanically deboned poultry meat (MDPM). The raw material obtained has a fine consistency and relatively low cost, with good nutritional value and functional properties suitable for the formulation of comminuted meat products (Yuste and others 1999; Sousa and others 2003; Pettersen and others 2004; Serdaroğlu and others 2005).

This process alters the original raw material composition, resulting in a material with higher fat and mineral content, by incorporation of lipids and heme pigments from marrowbone and the subcutaneous fat layer, besides calcium and phosphorus from bone particles (Sousa and others 2003). The main disadvantages of MDPM is the potential microbial load, which make it a highly perishable raw material (Yuste and others 1999). Also, the high heme pigments result in a darker colored product, susceptible to lipid oxidation (Lee and others 1997; Smyth and O'Neil 1997). In consequence, addition of MDPM affects physical, microbiological and sensorial properties of the product (Guerra-Daros and others 2005). These properties give MDPM particular characteristics as an ingredient in manufactured sausage.

80.2.1 Nutritional Properties

The nutritional quality of MDPM is comparable to normal poultry meat when evaluated by protein efficient ratio (PER) in rats. This suggests that MDPM may be beneficial in increasing the nutritional content of some processed poultry products because of MDPM's higher contents of calcium, phosphorus, iron, and other minerals (Babji and Yusof 1995). Reports have shown a high correlation between calcium and fluoride content in foods made with MDPM (Fein and Cerklewski 2001). As a result of inclusion of bone marrow in MDPM, there is a greater variation in the fatty acid content and higher percentage of cholesterol and phospholipids. Mechanical deboning increased monounsaturated fatty acids (MUFA), but had no effect on the percentage of polyunsaturated fatty acids (PUFA) (Serdaroğlu and others 2005). A major problem with products manufactured with MDPM is the rapid onset of oxidative rancidity, which results in off-flavor and odors, deteriorating sensory characteristics of sausages, even at frozen storage (Lee and others 1997; Pettersen and others 2004). On the other hand, sausages manufacture incorporating MDPM mixed with bovine plasma and red globules result in higher levels of isoleucine, which is considered a nonlimiting amino acid, with no detrimental effect on product acceptability (Benítez and others 2002). Table 80.1 compares the composition of deboned poultry meats (mechanically, manually and washed).

TABLE 80.1 Composition of Different Deboned Poultry Meats: Mechanically (MDPM), Manually Deboned (mDPM), and Washed Mechanically Deboned Poultry Meat (w-MDPM).

	MDPM	mDPM	w-MDPM
pH	6.3[a]	5.8[d]	6.5[a]
Humidity (%)	64–66[c,g]	74[d]	75–85[a,e]
Protein (%)	40–46[e]	20[d]	41–46[e]
Fat (%)	23[g]	14[d]	43[a]
Ash (%)	0.85[c]	1.0[d]	
SFA* (%)	36[d]	42[d]	
MUFA** (%)	30[d]	24[d]	
PUFA*** (%)	34[d]	33[d]	
MUFA/SFA	0.83[d]	0.58[d]	
PUFA/SFA	0.94[d]	0.79[d]	
Cholesterol (mg/100 g)	64[f]	57[f]	
EC (mL oil/g protein)	286–318[b]	217–268[b]	
WHC (g water/g protein)	2.59–3.07[b]	2.69–3.39[b]	

Source: [a]Smyth and O'Neil (1997); [b]adapted from Abdullah and Al-Najdawi (2005); [c]Guerra-Daros and others (2005); [d]Serdaroğlu and others (2005); [e]Yang and Froning (1992); [f]Babji and others (1998); [g]Mielnik and others (2002).

*Saturated fatty acids (SUFA).

**Monounsaturated fatty acids (MUFA).

***Polyunsaturated fatty acids (PUFA).

80.2.2 Microbiological Properties

Poultry meat and its derivates are among the food products that cause the most concern to Public Health authorities, owing to the associated risk of bacterial food poisoning (Beli and others 2001). *Salmonella*, *Listeria*, and *Staphylococcus* are the microorganisms most frequently associated with this kind of meat (Greenwood and Swaminathan 1981; Bijker and others 1987; Marinsek and Grebenc 2002). Contamination is the result of the lack of hygienic measures observed during boning of carcasses, collection, storage, and transport of bones or poultry parts. In this view, MDPM has to be employed in fully-cooked cured meat products, in order to reduce the chance of microbial contamination. Deterioration of sensory characteristics of MDPM sausages after 8 days of storage at 4°C were attributed to the increased psychotropic growth (Lee and others 1997).

80.2.3 Functional Properties

Muscle proteins are the principal functional and structural components of processed meats, determining product texture and appearance (Smith 1988). For MDPM, the functional properties of most interest are water retention and ability to emulsify fat (Guerra-Daros and others 2005). MDPM had a higher emulsion capacity than manually deboned poultry meat, although it presented no difference in water-holding capacity (Abdullah and Al-Najdawi 2005). In the same way, muscle protein solubility is markedly affected by the cations present in the meat system. Calcium chloride tends to enhance muscle protein solubility as compared to sodium chloride (Morrissey and others 1987), improving myosin extractability and solubility (Nayak and others 1996). Based on the relatively high calcium content and great emulsifying properties, MDPM is a better raw source of low-cost muscle proteins for sausage manufacture, as compared to other kind of poultry meat. Also a partial proteolysis of MDPM improves protein functionality (Smith and Brekke 1985).

80.3 POULTRY SAUSAGE MANUFACTURE

Sausage manufacturing includes a consecutive series of steps, including extract and solubilized muscle proteins, to emulsify fat and form a protein gel matrix. The final gel lattice is responsible of water and fat retention, texture, and juiciness in the final product. MDPM presents some advantages for these types of products. Finely comminuted meat products, such as frankfurters, hot dogs, and bolognas, are made from meat that is entirely or partly disrupted under the presence of water, salt and other processing ingredients. Meat had to be ground and comminuted in order to extract muscle proteins. The higher the degree of comminuting, the larger the amount of extracted protein (Hoogenkamp 1995). The use of MDPM saves this step in sausage manufacture due to its finely ground texture.

The mechanical agitation of the mixer or cutter of the grounded or chopped lean meat with salt and water enhances salt activation of the proteins, initiating the formation of a lean meat lattice (Terrel 1980). Solubilization of protein in salt solutions is an important physicochemical process for manufacturing of processed muscle foods (Xiong 1994). MDPM has a fine consistency and is practically ground; protein solubility is easily achieved during this step. Since MDPM is handled frozen and less energy is required to disrupt the tissue, a lower temperature during mechanical agitation can be achieved.

After proteins were solubilized, fat is added in the mixer or cutter, in combination with the seasonings and more water. Mechanical agitation entraps the fat into the lean lattice, and final fine-cut batter is packed in a casing, which physically restrains the lattice until proteins form a gel in a casing-shaped semisolid gel (Terrel 1980). Solubilized protein surrounds the fat and during thermal processing forms a network which prevents the coalescence of fat. This network also arranges the fixation or structural enforcement of all supporting compositional ingredients, such as meat and nonmeat proteins (Hoogenkamp 1995).

The percent of MDPM that can be included in formulation affects the final product characteristics (Lee and others 1997; Benítez and others 2002; Sousa and others 2003; Guerra-Daros and others 2005). In some cases MDPM's dark color can alter the sausage color (Smyth and O' Neil 1997; Yuste and others 1999; Perlo and others 2003; Serdaroğlu and others 2005) without the use of colorants in formulation. A surimi process in MDPM, however, can increase myofibrillar protein concentration and removal of color of the washed meat with an important dark red color diminution and an increase in lightness (Yang and Froning 1992; Smyth and O'Neil 1997; Perlo and others 2003). Alternative processes such as high pressure cooking enhances MDPM sausage texture, color and yielding (Yuste and others 1999). Although the inclusion of

TABLE 80.2 Mean Composition for MDPM Sausages (at Least with 80% of MDPM in Formulation).

Parameter	Range	Reference
Humidity (%)	66.3–69.1	Lee and others 1997; Babji and others 1998
Protein (%)	11.2–12.0	Babji and others 1998; Guerra-Daros and others 2005
Cholesterol (mg/100 g)	78.71	Babji and others 1998
Ashes (%)	1.16–2.3	Babji and others 1998
Iron (mg/100 g)	1.5–3.1	Sousa and others 2003
Calcium (mg/100 g)	75–141	Sousa and others 2003
Fluoride (mg/100 g)	0.15–0.22	Guerra-Daros and others 2005

MDPM in sausages resulted in a decrease in protein content, myosin, reducing tensile, and compression strength due to the lower myofibrillar content of meat (Guerra-Daros and others 2005), this detrimental effect of MDPM in sausage structural integrity can be compensated with the addition of other nonmeat proteins. Table 80.2 presents the main characteristics of MDPM sausages.

80.3.1 Effect of Gelling Polysaccharides on MDPM Sausages Texture

Low cost and functional properties of MDPM represent a great advantage over other raw meat protein sources. The interaction of MDPM proteins and gelling polysaccharides can be employed as an alternative to reduce fat in poultry sausages. For example, low-fat (5% w/w) poultry sausages were manufactured employing MDPM (Comercializadora Avemex, SA de CV, México) and ground poultry meat (leg and thigh). Three different polysaccharides were employed: sodium alginate (ALG) and κ-carrageenan (KCG) and gellan gum (GLN) with 2% of NaCl, adjusting the formulation with ice. A textural profile analysis (TPA) was performed to 20 mm height samples (20 mm Ø) at a constant speed of 1 mm/s in a TA-HDi texture analyzer (Texture Technologies, Scarsdale, New York/Stable Micro Systems, Surrey, United Kingdom), equipped with a 25 kg load cell and a 25 mm diameter acrylic probe. TPA parameters, that is, hardness, cohesiveness, springiness, and resilience, were calculated in a double compression force-deformation curves (Bourne 1978; Texture Technologies 2005).

Poultry sausages manufactured with MDPM were less hard than the ones made with poultry meat. Similarly, both sausages containing KCG resulted in a harder product

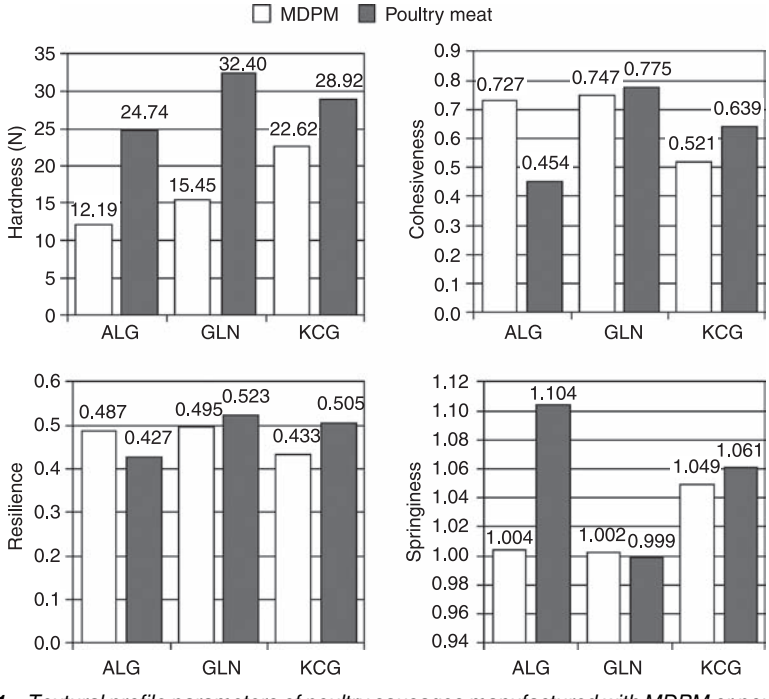

Figure 80.1 *Textural profile parameters of poultry sausages manufactured with MDPM or poultry meat and mixed with sodium alginate (ALG), gellan gum (GLN), or κ-carrageenan (KCG).*

than GLN or ALG sausages. Sausages made with MDPM and ALG resulted in a more cohesive product than the sausages made with poultry meat, with no significant difference between both MDPM or poultry meat mixed with GLN or KCG. No difference was detected in samples resilience. Poultry meat sausages with ALG had higher springiness values than MDPM sausages, but no difference was observed between the types of poultry meat with the same gum (Fig. 80.1). The main difference in texture can be explained by the effect of different cations on the gelling properties of the polysaccharides or gums employed, such as sodium alginate (Clare 1993), κ-carrageenan (Therklensen 1993) or Gellan (Sworn 2000). The presence of calcium in MDPM is supposed to be high however, the availability to interact with these gums may be limited. Only samples of MDPM with sodium alginate (ALG) presented a more compact and dense structure. The cohesiveness and resilience of these samples were higher than poultry meat-ALG sausages, with the interrelated lower springiness. With lower fat sausage formulation, the relatively higher fat content of MDPM could explain no differences in texture. This maybe due to the effect of the calcium present in this kind of meat over the gelling polysaccharides employed, making MDPM a good alternative from both nutritional and functional points of view for sausage manufacturing.

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81

Flavor of Fresh and Frozen Poultry

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81.1 Introduction 784
81.2 Poultry Processing 784
81.3 Fresh and Frozen Poultry 786
81.4 Basis of Poultry Meat Flavor 787
81.4.1 Maillard Reaction 788
81.4.2 Lipid Oxidation 789
81.4.3 Cholesterol Oxidation 789
81.5 Processing Factors Related to Poultry Flavor 790
81.5.1 Genotype and Age 790
81.5.2 Feeding 790
81.5.3 Antioxidants 791
81.5.4 Slaughtering and Postmortem Factors 792
81.5.5 Frozen Storage 792
81.5.6 Packaging 793
81.6 Conclusions 793
References 793

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81.1 INTRODUCTION

Poultry is derived from the skeletal muscles of various species of birds that have been domesticated to reproduce and grow in captivity, accompanied by a high animal feed to meat conversion ratio and an ability to survive under extreme temperatures. Chickens and turkeys are the most common types of poultry; other commercially available species are ducks, geese, pigeons, quails, pheasants, ostriches, and emus (Parkhurs and Mountney 1988). Poultry consumption has risen considerably in the last two decades, with an annual growth rate close to 10% (Table 81.1), and estimates indicate that production will continue to rise at the expenses of red meat, mainly because of health and food-safety concerns, such as the recent international problems with BSE, foot and mouth disease, dioxin-tainted products, and cholesterol and related cardiac problems.

In general, poultry meat is a good source of protein, fat, vitamins, and minerals in the diet (Table 81.2 shows the composition of various poultry cuts). Moreover, poultry is considered healthier than other, red meats, as it is lower in fat and cholesterol, higher in protein, and white, with good flavor qualities; it is also accepted by most cultures, and lower in cost when compared to red meats and fish. Flavor quality is affected by several factors, including antemortem determinants occurring over the bird's life, such as breed, physiology, nutrition, or diseases, which affect the composition of raw meat and therefore availability of flavor precursors. In addition, harvesting, slaughtering, and postmortem handling also have a great influence on poultry meat quality and flavor stability. The aim of this chapter is to review production and processing factors that influence flavor quality of fresh and frozen poultry.

81.2 POULTRY PROCESSING

The first stage of poultry meat production involves conversion of a live animal into meat; it is critical to avoid microbial contamination all the way from slaughtering until chilling or freezing, in order to maximize food quality for marketing and further processing. When

TABLE 81.1 Broiler Consumption Summary (1000 Metric Tons, Ready to Cook Equivalent).

	2000	2001	2002	2003	2004(f)	2005(p)
China	9,393	9,237	9,556	9,963	9,670	9,990
European Union	6,934	7,309	7,108	7,064	7,195	7,270
Brazil	5,110	5,341	5,872	5,742	5,850	6,140
Mexico	2,162	2,310	2,424	2,626	2,755	2,883
India	1,080	1,250	1,400	1,600	1,650	1,800
Japan	1,772	1,797	1,830	1,841	1,645	1,660
Russian Fed.	1,320	1,588	1,697	1,680	1,584	1,585
Canada	980	925	928	939	980	982
Malasya	812	846	821	868	881	942
Saudi Arabia	815	884	870	873	890	913
EUA	11,474	11,558	12,269	12,539	13,305	13,748
Others	37,890	7,629	7,565	6,855	6,692	7,082
World total	49,364	50,674	52,340	52,590	53,097	54,995

USDA, Foreign Agricultural Service, data created: 11/2/2004.
P, Preliminary; f, forecast.

TABLE 81.2 Composition of Poultry Cuts (per 100 g).

	Energy (kcal)	Fat (g)	Protein (g)	Cholesterol (mg)	Iron (mg)	Niacin (mg)	Thiamine (mg)
			Chicken				
Light meat with skin	186	11.07	20.27	67	0.79	8.9	0.059
Dark meat with skin	237	18.34	16.69	81	0.98	5.2	0.061
Light meat without skin	114	1.65	23.2	58	0.73	10.6	0.068
Dark meat without skin	125	4.31	20.08	80	1.03	6.2	0.077
MDP with skin	272	24.73	11.39	130	1.57	4.6	0.050
MDP without skin	199	15.48	13.79	104	1.73	6.2	0.071
			Turkey				
Light meat with skin	159	7.36	21.64	65	1.21	5.1	0.056
Dark meat with skin	129	4.79	20.06	87	1.62	2.8	0.046
Light meat without skin	114	1.57	23.43	60	1.01	5.8	0.064
Dark meat without skin	111	2.67	20.46	81	1.66	2.9	0.049
MDT	201	15.96	13.29	95	1.61	1.9	0.048

Source: USDA National Nutrient Database for standard reference, release 17 (2004).

birds reach "harvest" time, they are generally taken off feed and water to empty their digestive tract and reduce the potential for contamination during processing. Birds are placed into plastic or wooden transport cages and then transported to the slaughterhouse. In the next step, birds are removed from the cages and transferred to continuously moving shackles where they are suspended by both legs. Birds are usually stunned by running their heads through a water bath that conducts an electric current to produce unconsciousness, and killed either by hand or by a mechanical rotary knife, which cuts the jugular veins and the carotid arteries at the neck, after which they are left to bleed. Following bleeding, birds go through scalding tanks of hot water (50–60°C), which softens the skin, so that the feathers can be removed efficiently. Carcasses then go through feather-picking machines equipped with rubber "fingers" designed to beat off the feathers, then pass through a flame that burns off any remaining feathers. Finally, heads, legs, and viscera are removed. Carcasses are generally inspected during the evisceration process. The inspection procedures in the poultry industry vary around the world and may be performed by government inspectors, veterinarians, or plant personnel, depending on the country's laws. After carcasses have been washed, they are chilled to a temperature below 4°C. Usually, carcasses are cut up into pieces and the frame, back, neck, drumsticks, and wings are often mechanically separated. Carcasses and cut-up pieces must be chilled immediately after slaughter, reaching 4°C within 4 h of death for chickens and 8 h for turkeys. The two common methods for chilling include immersion in an iced water bath, or air chilling in chambers with circulating cold (−7 to 2°C) air for 1 to 3 h (Sams 2001).

Boneless cuts and poultry pieces can be iced in combos or boxes and held in a cooler at −2.2°C up to 24–36 h prior to being processed. On the other hand, poultry pieces or mechanically deboned poultry (MDP) are usually boxed in plastic-lined or waxed cardboard containers (to exclude air and protect the surface from excessive drying) and frozen immediately. Water chilling is used throughout North America and involves a prechilling step in which a countercurrent flow of cold water is used to lower the temperature of carcasses and to minimize bacterial contamination by diluting microorganisms into the water tank. At the entrance of the prechiller, the carcass temperature is about 38°C, and

lipids are still fluid. Water penetrates into the skin and other subcutaneous tissues, leading to an increase in poultry weight. The amount of water gained is carefully regulated, in the United States the legal limits for water pickup are 8% for birds going directly to market and 12% for birds to be further processed. After prechilling, carcasses enter the main chilling tank, which rapidly reduces the temperature to 4°C, and temperature declines, lipids solidify, which stops the water being absorbed. The air-chilling method is the standard in Europe; carcasses are hung by shackles and moved through coolers with rapidly moving air. The process is less energy-efficient than water chilling, and the birds may lose weight due to dehydration. Air chilling prevents cross-contamination between birds. However, if a single bird contains a high number of pathogens, this pathogen count will remain on the bird. The final temperature of the carcasses before shipment is usually about −2 to −1°C, just above the freezing point for poultry. In some cases a slight crusting on the surface occurs during the final chilling. For water-chilled carcasses this final chilling takes place after packaging, when the carcasses are placed in an air chiller (Sams 2001).

81.3 FRESH AND FROZEN POULTRY

Raw materials include fresh chilled or frozen skeletal muscles such as boneless breasts, legs, thighs, desinewed drumsticks, and mechanically deboned poultry meat (MDP), with or without skin. Whole or individual parts of birds may be packaged raw for direct sale. Poultry provides an excellent medium for the growth of spoilage and pathogen microorganisms such as Pseudomonas, Staphylococcus, Micrococcus, Acinetobacter, Moraxella, and Salmonella. Contamination of poultry takes place during the slaughtering and processing procedures, by contact of the carcass with feathers, feet, or intestinal contents, which have a high microbial load, and also because of the use of contaminated equipment and physical manipulation during deboning and grinding. Prevention of microbial contamination involves careful regulation and monitoring of slaughtering and processing plants, proper handling, and storage at low temperature. Poultry, similar to other meats, is very perishable if stored under ambient conditions, and storage under low temperature such as refrigeration and freezing extends storage life.

A large portion of poultry is used as a basic raw material to manufacture ready meals; where it can be supplied as boneless or mechanically deboned frozen meat blocks and is used in the meat industry. Freezing is widely used in the poultry meat industry as the safest and most economical preservation method, as it prevents microbial spoilage and minimizes the rate of biochemical reactions in muscle for several months. Freezing is also used in the household as a common storage practice for raw poultry between purchasing and cooking, as well as for precooked and ready-to-eat frozen chicken products. However, undesirable quality changes do occur during frozen storage such as texture toughening, color darkening, and off-flavor development, which all reduce its acceptability and shelf-life. Therefore, poultry packages must include instructions about safe handling.

Most raw turkey is sold frozen, but most chicken is sold fresh. Poultry carcasses are generally cut into a number of pieces, which are placed on plastic foam trays and covered with a plastic film. A "diaper" (absorbent paper) is often used to catch any liquid that may be released from the meat. Fresh poultry should be used within 14–21 days after slaughter and generally should not be kept in the home refrigerator for more than three days. In the United States, poultry that has been frozen to a temperature of −5° to −4°C is allowed to be thawed and sold as "fresh." Frozen poultry is usually vacuum-packed in plastic bags and then frozen in high-velocity freezers. Before freezing,

the poultry carcass may be injected with various salts, flavorings, and oils in order to increase the juiciness of the meat. Injections are usually done with a multineedle automatic injector, and information about the added ingredients is indicated on the package label. Frozen storage time for poultry products (including poultry bought fresh and frozen in a home freezer) depends on the temperature of the freezer, the quality of the packaging, and the cycling of the freezer; frozen products should be used within three months.

Chilling, and frozen storage with subsequent thawing, may decrease the levels of some poultry components such as proteins and minerals due to drip loss. Decreases in thiamin, riboflavin, and niacin have also been reported for dark meat; in particular, thiamin is very sensitive to freezing and can be destroyed during extended frozen storage (Hettiarachchy and Gnanasambandam 2000). For storage up to 6–10 months, frozen materials must be held at between $-17^{\circ}C$ and $-28.9^{\circ}C$, avoiding temperature changes, as this causes large ice crystals to grow in the muscle cells and excessive drip water to appear during thawing. Fresh cuts should not be stored above $4.4^{\circ}C$ when received, and frozen materials should be kept below $-17.8^{\circ}C$. Higher temperatures or temperature abuse in transit or processing means a potential decrease in shelf-life or a potential pathogen risk and off-flavor formation. Therefore, it is imperative to monitor temperature during all stages of processing, including transit and storage. Raw materials that show browning or graying may indicate prolonged storage, temperature abuse, or early microbial spoilage; greening, slime formation, putrefaction, souring, or other off-flavor are signs of apparent spoilage and therefore must be avoided (Mandava and Hoogenkamp 1999). Storage under low temperature slows most chemical and biochemical reactions with an increase in shelf-life. However, even under freezing conditions, solutes present in the unfrozen phase are in close proximity and some rate reactions such as lipid oxidation may increase. Therefore, lipid autoxidation is one of the most important chemical reactions to reduce shelf-life during frozen storage (Ang 2000; Shahidi 2000; Ponce-Alquicira 2004).

81.4 BASIS OF POULTRY MEAT FLAVOR

Flavor is one of the most important attributes to influence poultry meat acceptability; it is the result of taste odor and textural sensations perceived in the oral and nasal cavities. Compounds responsible for taste are nonvolatile at room temperature; they interact with the taste receptors located in the taste buds of the tongue. Taste is usually described with five basic tastes (salty, acid, sour, sweet, bitter and umami). While, aroma comprises a large amount of volatile compounds that are transported through the throat to the olfactory receptors located in the nose where they are sensed as acid, brothy, chickeny, fruity, and so on (Farmer 1999; Ang 2000; Ponce 2004).

Raw poultry, like other meats, has a metallic bloody flavor. However, during heating and storage, various chemical reactions take place between precursors, resulting in the production of numerous volatile compounds responsible for the poultry meat aroma. The main poultry flavor precursors are water-soluble compounds and lipids. The major reactions involved in the generation of poultry flavor include nonenzymatic browning (or the Maillard reaction), Strecker degradation, lipid oxidation, thiamin degradation, and proteolysis, among others (Farmer 1999; Ang 2000).

Free sugars, sugar phosphates, nucleotide-bound sugars, free amino acids, peptides, nucleotides, and other nitrogenous components such as thiamin are among the main water-soluble flavor precursors. All are present in the raw meat, but frozen storage and cooking may change their concentration because many of these substances are also precursors for

the aroma compounds. Interaction of nonvolatile compounds and/or their breakdown products generated during cooking and storage produce a large number of intermediates and volatiles that contribute to flavor. For example, ribose is one of the main sugars present in ribonucleotides such as adenosin triphosphate (ATP), which is essential for muscle function; during postslaughter, it is converted to inosine monophosphate (IMP) (Mottram 1998). Also, carnosine, an imidazole dipetide (α-alanine-L-histidine) occurs naturally in meat. Its concentration varies with species, from 50 to 276 mg/100 g in chicken leg and swine shoulder meat, respectively. This dipeptide has a buffer capacity and a strong antioxidant activity that reduces lipid oxidation. However, it is also the nitrogen source for pyrazine and thiazole generation, giving the roasty and nutty poultry flavor notes during cooking (Chen and Ho 2002). Carnosine executes its antioxidant activity by acting as a hydrogen donor and metal-chelating agent; however, the antioxidant activity varies according to concentration. At high levels (1.5%) it is a better hydrogen donor than α-tocopherol and BHT (Cuppet 2001).

Deteriorations in flavor, such as the appearance of rancidity, bitterness, or undesirable taste, are due to the formation of low-molecular-weight compounds from lipid oxidation or protein degradation and can take place during storage. Compounds derived mainly from lipids such as aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, esters, lactones, and alkylfuranes may lead to rancid off-flavors in both cooked and raw meat during long-term storage. Poultry and pork meats have a large proportion of unsaturated fatty acids, when compared with beef, and generate more unsaturated volatile aldehydes (as shown in Table 81.3). Unsaturated fatty acids undergo autoxidation much more readily than those that are saturated; therefore, triglycerides and structural phospholipids present in lean tissue are an important source of flavor compounds. Phospholipids are essential structural components of all cells and contain a higher proportion of unsaturated fatty acids than triglycerides, such as arachidonic acid ($C_{20}H_{32}O_2$) present in phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositides. This makes them more susceptible to oxidation and they have been associated with "warmer-over" flavor in reheated cooked meats (Mottram 1998). Grinding disrupts muscle tissue and allows the contact of cell contents with oxygen and the access of pro-oxidants to the more unsaturated fatty acids in the membrane phospholipids. During storage, reducing agents such as ascorbate and the superoxide anion can release the iron from myoglobin and ferritine, which is very active as a pro-oxidant against unsaturated fatty acids (Cuppett 2001).

81.4.1 Maillard Reaction

Another chemical reaction that causes major food flavor formation is nonenzymatic browning, also known as the Maillard reaction. This reaction takes place between reducing

TABLE 81.3 Lipid Composition of Meat (Value per 100 g of Edible Portion).

	Total Fat (g)	Total Fatty Acids			Cholesterol (mg)
		Saturated	Monounsaturated	Polyunsaturated	
Lean beef	5	2.25	2.151	0.257	62
Lamb	17.17	6.63	8.46	1.06	156
Pork	12.59	3.57	6.289	1.092	88
Chicken light meat	1.65	0.44	0.39	0.37	58
Turkey light meat	1.57	0.5	0.28	0.42	60

Source: USDA National nutrient database for standard reference, release 17 (2004).

sugars and a free amino group on amino acids or proteins present in foods. Products of the Maillard reaction lead to a darkening of color, reduction in protein solubility, development of bitter flavors, and loss of essential amino acids such as lysine. Flavor compounds generated include heterocyclic compounds, carbonyls, pyrazines, and furans, among others. The rate of this reaction is influenced, by water activity, temperature, and pH (Jousse and others 2002).

81.4.2 Lipid Oxidation

The fat content of poultry differs from that found in red meat (Table 81.3). Poultry has a higher proportion of unsaturated fatty acids; both turkey and chicken contain about 30% saturated, 43% monounsaturated, and 22% polyunsaturated fatty acids (Hettiarachchy and Gnanasambandam 2000). The high levels of unsaturated fatty acids make poultry more susceptible to rancidity through the oxidation of the double bonds in the unsaturated fatty acids, as saturated fatty acids are more resistant to oxidation. However, this fatty acid ratio has led to the suggestion that poultry may be a more healthful alternative to red meat. In birds, fat is primarily deposited under the skin or in the abdominal cavity; therefore, a significant amount of the fat can be removed by removing the skin before eating (Shahidi 2000).

The unsaturated fatty acids present in poultry meat are susceptible to autoxidation when exposed to oxygen. Unsaturated fatty autooxidation is autocatalytic and proceeds by a free-radical chain reaction. Free radicals contain an unpaired electron, and are capable of reacting energetically and indiscriminately with various compounds and initiate nonspecific hydrogen absorption and chemical addition; therefore, free radicals are highly reactive chemical molecules. The basic mechanisms in a free-radical chain reaction involve initiation, propagation, and termination steps. The initiation reaction comprises a free-radical molecule ($X^{\bullet}$) present in the food that removes a hydrogen (H) atom from a lipid molecule, producing a lipid radical ($L^{\bullet}$). This lipid radical reacts with molecular oxygen (O_2) to form a peroxy radical ($LOO^{\bullet}$). The peroxy radical removes a hydrogen atom from another lipid molecule and the reaction starts over again in the propagation stage. During the propagation steps, hydroperoxide molecules (LOOH) are formed that may break down into alkoxy ($LO^{\bullet}$) and peroxy radicals plus water (H_2O). The lipid, alkoxy, and peroxy radicals may combine to form stable, nonpropagating products (termination). Peroxides are unstable and will decompose to acids, alcohols, aldehydes carbonyls, and ketones, which results in the development of rancid off-flavors. In addition to promoting rancidity, the free radicals and peroxides produced in these reactions may have other negative effects, in color and destruction of vitamins A, C, and E. Lipid oxidation is catalyzed by ultraviolet light, heat, high pressure, metals, and oxygen (Belitz and Grosch 1999; Schmidt 2000). In addition, the low water activity found in frozen poultry meat also promotes lipid oxidation. Storage time and temperature have a significant influence on the formation of secondary lipid oxidation products, such as hexanal, octanal, and nonanal. At low refrigeration and freezing temperatures, lipid oxidation develops slowly, but can alter the flavor profile (Duncan 2000; Wiggers and others 2004).

81.4.3 Cholesterol Oxidation

Cholesterol is found in the cell membrane and is associated with polyunsaturated fatty acids of the membrane associated phospholipids. Oxidation of cholesterol occurs easily

in foods and is promoted by prolonged storage, application of heat, exposure to air, light, or irradiation; it is affected by the same factors involved in lipid oxidation and is closely related to the neighboring fatty acids, generating a series of oxidation products (COPs) such as 7-ketocholesterol (7-k). There is a great interest in cholesterol because it is associated with cardiovascular diseases, and also because its oxidation products have adverse effects such as atherogenesis, cytotoxicity, mutagenesis, carcinogenesis, and Alzheimer's disease. Cholesterol oxidation proceeds via a free radical mechanism, similar to polyunsaturated fatty acid oxidation, thus the use of antioxidants may prevent or retard cholesterol oxidation; however, results are diverse and sometimes contradictory. BHT and TBHQ are more effective inhibitors than BHA, while PG is unable to inhibit the synthesis of COPs. In addition to synthetic antioxidants, it has been claimed that tocopherols exhibit different antioxidant effect, where g-Tocopherol acts as the strongest antioxidant, followed by a-tocopherol. d-Tocopherol and b-tocopherol. Also, rosemary extract obtained from the leaves of *Rosmarinus officinalis. L*, that contains four antioxidants (carnosol, rosmanol, isorosmanol, and rosemarydiphenol) show some antioxidant cholesterol activity. (Lercker and Rodríguez-Estrada 2000; Lee and others 2001; Valenzuela and others 2003; Ponte and others 2004).

81.5 PROCESSING FACTORS RELATED TO POULTRY FLAVOR

Flavor quality is affected by several factors, including antemortem determinants that take place over the bird's life, such as breed, physiology, nutrition, or diseases. In addition, slaughter, postmortem handling, and storage conditions could affect the composition of raw meat and therefore availability of flavor precursors. Some factors related to fresh and frozen poultry processing are discussed below.

81.5.1 Genotype and Age

There are contradictions in the literature on the effect of genotype; however, reports regarding flavor differences between genotypes having different grow rates claim that those differences were mainly due to differences in age and body weight. In general, breast meat from older birds received higher scores in flavor than those from chickens slaughtered at younger ages. Chickens undergo physiological changes as they grow older and reach maturity, which may affect flavor precursors, as precursors may be present in higher concentrations in older chickens. There is a high genetic correlation between abdominal fat production and deposition that influences flavor (Farmer 1999; Zerehdaran and others 2004).

81.5.2 Feeding

The phospholipid profile is closely influenced by the fat composition of feed. Thus, the way in which a bird is fed can influence meat quality. The type of fatty acid composition in poultry and its stability are correlated to the fatty acid composition of ingested fats. Meat composition can be modified by diet; feeding of fish oils or highly unsaturated vegetable oils is known to produce fishy flavors in the meat (Farmer 1999). Birds fed on diets rich in unsaturated fats have an increase in the polyunsaturated fatty acid content of their lipid fraction. This modification is nutritionally desirable, but increases susceptibility of meat to oxidation. However, dietary supplementation with antioxidants such as

tocopherols, carotenoids, and ascorbic acids supplements is recommended to avoid discoloration and improve oxidative stability of cooked and raw poultry meat during frozen storage (James 2000; Grau and others 2001). Bou and others (2004) supplied diets with 2.5% fish oil and produced chicken meat with eicosapentaenoic and docosahexaenoic acid contents double those of diets supplied with 1.25% fish oil. Also, consumer acceptability revealed no variation after 5 months of storage at $-20^{\circ}C$ in comparison with fresh commercial chicken meat used as blind control. Cortinas and others reported similar data (2004). Inclusion of dietary polyunsaturated fatty acids (PUFA) also increased the level of fatty acid in meat; however, increasing the PUFA content decreased total fatty acids in thigh and had no effect in breast meat. In addition, Du and others (2001) fed chicken with conjugated linoleic acid (CLA), which is easily incorporated into tissue and egg, increasing storage stability with respect to oxidative changes in the meat, due to an increase in saturated fatty acids and CLA. The dietary carbohydrate source also appears to affect meat flavor and general acceptance of broiler meat. Some reports indicate that broilers on rations containing dairy products produced meat with more flavor, and corn-fed poultry was more flavorful than poultry fed barley, oats, milo, and wheat (Lyon and others 2004).

81.5.3 Antioxidants

Antioxidants can react with peroxyl or alcoxyl radicals and terminate the chain reaction or peroxidation by scavenging chain-propagating radicals and prevent lipid oxidation. The use of antioxidants such as gallate, sesamol, tocopherols, and carotenoids, added directly to meat or via dietary supplementation, increases the oxidative stability of muscle foods (Lee and Ahn 2003). The activity of endogenous antioxidant enzymes (catalase, superoxide dismutase, and glutation reductasa) seems to be directly related to the dietary polyunsaturated fats; however, contradictory data have been reported regarding the activity of these antioxidant enzymes.

Vitamin E compounds (tocopherols and tocotrienols) are well recognized for their effective inhibition of lipid oxidation in food and biological systems. There are many data that have proved the effect of tocopherol and carotenoid diet supplementation is increasing the oxidative stability of poultry meat. Dietary natural antioxidants may be better alternatives to direct addition after slaughtering, as it ensures an even distribution of antioxidants in the meat. In addition, those natural antioxidants bring health benefits in disease conditions such as cancer, coronary heart disease, and those related to immune functions (Kang and others 2001).

When tocopherol is added via the diet in the range 25–2000 IU/kg, it is incorporated into the subcellular membrane and intercepts free radicals as they are formed in both dark and white broiler meat. Also, supplementation of vitamin E from 200 up to 600 IU/kg and β-carotene alone may increase oxidation in meat, even though this practice is very common as it gives a golden tone desirable by consumers. Some level of tocopherols needs to be added to carotenoids to act as antioxidants in raw and cooked meat; the tocopherols act as a hydrogen donor and canthaxantine stops the peroxy free-radical chain propagation by trapping the radical in its conjugated polyene systems. Reports indicate that vitamin E and carotenes have a higher effect on poultry flavor than dietary fat (Ruiz and others 2001).

Low concentrations (200–300 ng/kg of meat) of ascorbic acid in meat lead to a prooxidant effect by reducing free transition metals such as Fe(III) or Cu(II) to Fe(II) and Cu(I), at which catalysis of lipid hydroperoxide decomposition to free radicals is more active. In

contrast, at higher concentrations, ascorbic acid acts as an antioxidant because of its ability to scavenge oxygen and lipid free radicals. Amounts of ascorbic acid produced by self-synthesis in chicken meat vary from 40 to 65 mg/kg. Supplementation effectively increases the levels of ascorbic acid in meat; however, levels decrease rapidly after removal of dietary supplementation. Additionally, ascorbic acid is readily oxidized by heat in the presence of metal ions at very low levels of oxygen (Grau and others 2001).

81.5.4 Slaughtering and Postmortem Factors

In general, meat flavor improves during postmortem ageing of meat. When animals die, muscle cells remain active, continuing their metabolism by using energy stored in them. With the loss of blood and oxygen supply, muscle cells gradually shift from aerobic metabolism to anaerobic. Accumulation of lactic acid derived from the anaerobic metabolism causes the pH to decrease from near neutrality to a pH value close to 5.7. This pH reduction reduces the activity of some ATP-producing enzymes; therefore, ATP muscle concentration falls to levels below 1 μM/g, inducing the severe muscle contraction known as rigor mortis. The breakdown products of lipids, proteins, and nucleic acids, which are formed by the action of indigenous meat enzymes during the ageing process, are likely to be related to the flavor precursors. Lysosomal enzyme acid phosphatase activity decreases rapidly during postmortem ageing, but cathepsin B and D activities continue, leading to formation of small peptides and free amino acids that act as flavor enhancers (Farmer 1999; Liu and others 2004).

81.5.5 Frozen Storage

The shelf-life of poultry is increased to a large extent by frozen storage at -18°C to -20°C. Flesh can be directly frozen in one single step or cooled and then frozen using an air blast freezer (with an air stream at -40°C, 3–10 m/s). Freezing involves removal of heat accompanied by a phase change, converting water to ice; however, in the remanding unfrozen water, the concentration of dissolved compounds increases and water activity decreases. Although the physicochemical reactions slow down at low temperatures, they will continue during frozen storage. Therefore, the shelf-life of frozen chicken is affected by storage temperature, and deterioration is mainly due to oxidative changes in the lipids. Poultry is more susceptible than pork, beef, or mutton (Belizt and Grosch 1999; Zaritzky 2000). Lipid oxidation is one of the main issues limiting the quality and acceptability of poultry and poultry products. This process affects lipids, pigments, proteins, carbohydrates, and vitamins, causing discoloration, drip losses, off-flavor development, and loss of nutrient value. The susceptibility to lipid oxidation depends on several factors such as the level of polyunsaturated fatty acids present in meat, preslaughter events such as stress, postslaughter handling, storage temperature, pH, and disruption of muscle membranes and cooking. Catalysis of lipid oxidation can occur by the action of low-molecular-weight metals and iron-containing heme proteins. Disruption of lipid membranes, as in mechanically separated poultry meat, facilitates the interactions of pro-oxidants such as oxygen with unsaturated fatty acid, resulting in the generation of free radicals and the propagation of oxidative reactions (Petterson and others 2004). Secondary lipid oxidation products such as α- and β-unsaturated aldehydes are more polar than their parent compounds and can diffuse from membranes into the surrounding sarcoplasm, interacting with sarcoplasmic proteins including myoglobin, which also act as

a prooxidant (Fausman and others 1999). Poultry meat contains more polyunsaturated fatty acids than red meat and can be more susceptible to oxidative changes (Kilic and Richards 2003).

81.5.6 Packaging

Packaging materials protect and preserve products during storage and distribution. Polymers used for food packaging include polyolefins such polyethylene (PE) and polypropylene (PP) because of their good heat sealability and excellent moisture resistance; however, those materials can absorb low-molecular-weight compounds and flavors. Poultry meat may undergo loss of quality due to failure of the package and because of product–package interactions, as packaging is used in direct contact with meat and adsorption of flavor compounds may alter the aroma and taste. Van Willige and others (2002) demonstrated that the rate of flavor absorption of polyolefins (PE, PP) is 3–2400 times higher than polyesters (polycarbonate, polyethylene terepthalate, and polyethylene naphathalate) and that absorption increases with temperature from 4 to 40°C. Therefore, fresh poultry is more susceptible to the changes generated by product–package interactions.

It is unavoidable that certain water evaporation from the meat surface takes place during frozen storage, resulting in quality and weight losses. Surface of meat improperly packaged will dehydrate, developing the commonly called freezer burn, which has a brown appearance and quickly leeds to rancidity, thus altering the quality and flavor. Only if the product is tightly packaged in a water vapor impermeable film, can evaporation be completely avoided, but if there are small spaces between the product and the package material, ice is deposited there (Dawson 2001).

81.6 CONCLUSIONS

Poultry is a very popular source for protein; however, flavor quality can be affected by several factors, including both ante-mortem and post-mortem conditions that influence raw meat composition and therefore availability of flavor precursors. Understanding of mechanisms involved in poultry flavor formation and improvement of storage conditions for fresh and frozen poultry will guarantee the acceptability and the continuous consumption increment.

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82

Edible Packaging for Poultry and Poultry Products

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82.1 Introduction 798
82.2 Definition and History Aspects of Edible Packaging 799
82.3 Importance of the Application of Edible Films and Coatings in Meat and Poultry 799
82.4 Types of Edible Films: Characteristics and Some Applications 800
82.4.1 Lipid Based Films 800
82.4.1.1 Waxes 801
82.4.1.2 Glycerides 801
82.4.2 Polysaccharide Based Films 801
82.4.2.1 Alginates 801
82.4.2.2 Carrageenan 802
82.4.2.3 Agar 802
82.4.2.4 Starch 802
82.4.2.5 Chitosan 802
82.4.2.6 Cellulose and Cellulose Derivates 803
82.4.3 Protein Based Films 803
82.4.3.1 Gelatin 803
82.4.3.2 Corn Zein 803
82.4.3.3 Soy Protein Isolates 804
82.4.3.4 Whey Protein Isolate 804
82.4.4 Composite Films 804
82.5 Mechanisms to Manufacture Edible Films and Coatings 805
82.6 Substances Used to Improve Mechanical Properties of Films 805
82.7 Methods for Application of Edible Films and Coatings 806
82.8 Safety and Health Considerations of Edible Films 806

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

82.9 Active and Edible Packaging 807
82.9.1 Carbon Dioxide Generating Systems 807
82.9.2 Ethanol Generating Systems 807
82.9.3 Antioxidants 807
82.10 Antimicrobial Food Packaging 808
82.11 Conclusions 811
References 811

82.1 INTRODUCTION

Due to their chemical composition, poultry meat and other muscle foods are extremely susceptible to suffer deterioration during storage. These modifications include changes in food texture, as well as loss of flavor, color, or nutritional value (Wu and others 2002). Additionally, poultry meat can be contaminated with pathogenic and spoilage microorganisms that accelerate deteriorative changes and are associated to food intoxication. Therefore, several methods of preservation such as freezing, curing, salting, heating, and others have been used to improve safety and maintain food quality. In response to the increasing demand for high quality ready-to-eat foods, food technologists have focused their attention on the development of mildly preserved foods that maintain their natural and fresh appearance as far as possible (Guilbert and others 1996).

Poultry meat shelf-life is reduced by contamination with chemicals and microorganisms present in the environment or by contact with contaminated surfaces during slaughtering, processing, and distribution. The use of packaging is the result of ancestral humankind's instinct to cover food and protect it from the environment, either by using fresh or dehydrated leaves, guts, cloth, baskets, and paper, among other materials (Miller and Krochta 1997). In 1950 heat shrinkable polyvinyl chloride (PVC) was introduced, and integrated into packaging films; nowadays, more than 90% of the poultry meat produced in the United States is marked under vacuum or modified atmospheric packaging (Cutter and Sumner 2002). However, food packaging is dominated by petroleum derivates polymers such as polyethylene and polystyrene (Cha and Chinnan 2004). These materials are nonbiodegradable and adversely impact the environment. Recently, the development of edible packaging in the form of films and coatings has received increasing interest in response to the tendency for reducing the amount of food packaging materials and/or substitute synthetic materials for biodegradable based packaging materials (Petersen and others 1999; Wu and others 2002). Additionally, edible films can be consumed along with the wrapped food providing additional nutrients, enhancing organoleptic properties, and offering protection even when the package has been opened (Guilbert and others 1996; Cutter and Sumner 2002).

However, edible films and coatings will never replace the nonedible packaging made from synthetic materials, even though edible packaging is made from natural biodegradable and renewable biopolymers. The utility of edible films depends on their capacity to:

- Improve overall foods quality;
- Extend shelf-life;
- Improve economic efficiency of packaging materials.

One obvious advantage is that edible films will reduce synthetic waste materials used in food packaging (Kester and Fennema 1986; Krochta and De Mulder-Johnston 1997).

Natural biopolymers for edible packaging include lipids, polysaccharides, and proteins. These materials can act as a barrier by limiting moisture, aroma, and lipid migration between food components, and their efficiency will depend on their chemical characteristics (Kester and Fennema 1986; Guilbert and others 1996; Gennadios and others 1997; Wu and others 2002). In addition to the barrier properties, edible films and coatings can be incorporated with functional additives such as antioxidants, nutrients, and antimicrobial agents (Wu and others 2002). This type of edible packaging is part of the classification of a new generation called "active packaging," which has been defined as "the packaging that changes its condition to extend shelf-life, improve safety and sensory properties while maintaining the quality"; making the antimicrobial food packaging the most promising form of active packaging (Quintavalla and Vicini 2002). This type of packaging has a great potential to be used for ready-to-eat and cured poultry products in response to the U.S. Department of Agriculture (USDA) policy of "zero tolerance" for *Listeria monocytogenes* (Zhu and others 2005), which may contaminate the products.

The aim of this chapter is to explore the importance of edible packaging, the materials and functionality, and their application in poultry meat preservation. In addition, special attention is drawn to analyze the advances in antimicrobial edible packaging to ensure meat safety.

82.2 DEFINITION AND HISTORY ASPECTS OF EDIBLE PACKAGING

Edible packaging can be applied on foods in the form of films or coatings. An edible film or coating can be defined as a thin layer of material that can be eaten by the consumer, providing barrier protection against microbial contamination and moisture losses, and extending shelf-life (Guilbert and others 1996; Wu and others 2002).

The use of edible packaging to extend the shelf-life of foods is not a new concept. In China, during the 12th and 13th centuries, coating of fresh oranges and lemons with wax was used to prevent desiccation (Kester and Fennema 1986). Later on, the application of lipid coatings was first documented in the 16th century (Cutter and Sumner 2002). However, patents on edible films to extend the food shelf-life date back to the 1950s, comprising films for frozen meat, sea foods, and poultry using alginates, fats, gums, and starches (Guilbert and others 1996). Currently, there are several reports where edible films are used to improve the sensorial and microbiologic quality of meats and poultry products (Herald and others 1996; Natrajan and Sheldon 2000a,b; Dawson and others 2002; Janes and others 2002; Lungu and Johnson 2005a,b; Theivendran and others 2006).

82.3 IMPORTANCE OF THE APPLICATION OF EDIBLE FILMS AND COATINGS IN MEAT AND POULTRY

According to Gennadios and others (1997), the potential benefits of using edible coatings in muscle foods such as poultry are:

1. Reduction in moisture loss during storage of fresh or frozen meats that leads to texture, flavor, and color changes while reducing weight.

2. Prevention of drip losses of fresh meats, and eliminating the need to use absorbent pads.
3. Reduction of the lipids and myoglobin oxidation rate, by using edible coatings with low oxygen permeability.
4. Reduction of the load of pathogenic and spoilage flora, and partial inactivation of proteolytic enzymes on the meat surface.
5. Restricting loss of volatile flavors and foreign odor pick-up.
6. Serving as carriers of antioxidants and antimicrobials in active packaging.
7. Reduction of oil uptake during frying.

Therefore, edible packaging applied as coatings on foods may also maintain the quality of foods after the package is opened, thus protecting against moisture change, oxygen uptake, and aroma loss (Krochta and De Mulder-Johnston 1997).

82.4 TYPES OF EDIBLE FILMS: CHARACTERISTICS AND SOME APPLICATIONS

Edible films and coatings can be made from lipids, polysaccharides, proteins, and composites (Kester and Fennema 1986; Gennadios and others 1997; Wu and others 2002). Each film type presents its own functional properties; some film characteristics are presented below in addition to examples of their application.

82.4.1 Lipid Based Films

Several film forming solutions prepared from waxes (carnauba, beeswax, and paraffin wax), oils (vegetal, animal, and mineral) and surfactants can be used as protective coatings (Kester and Fennema 1986; Gennadios and others 1997; Cutter and Sumner 2002). Coatings made from lipids can block moisture transport due to their relatively low polarity. In this context, wax coatings have shown to be substantially more resistant to moisture transport than other lipid or nonlipid edible coatings (Kester and Fennema 1986; Gennadios and others 1997). Hence, lipids are mainly used for their efficiency as a water–vapor barrier in edible films. Lipid characteristics such as structure, degree of saturation, chain length, physical state, and crystallization shape influence their film functional properties (Cutter and Sumner 2002). Nevertheless, lipid based films have rather poor mechanical properties (Yang and Paulson 2000).

The application of lipid coatings was first documented in the 16th century, when animal fats were used to coat meat cuts in order to prevent shrinkage; this process was called larding (Cutter and Sumner 2002). In 1955, McNally dipped whole dressed chickens into molten wax, mineral oil, corn oil, or lard prior to freezing (Gennadios and others 1997). Today animal fats are still used to coat a variety of foods, including frozen poultry. Frozen poultry did not undergo substantial dehydration when coated in oil–water emulsions prepared at 60–80°C by blending an animal fat or vegetable oil with emulsifiers, water, seasonings, and preservative agents (Gennadios and others 1997). Lipid materials have also been used in edible films to impart hydrophobicity, cohesiveness, and flexibility. However, lipid coatings can produce anaerobic conditions at higher storage temperatures (Cutter and Sumner 2002). If the film is formed from lipid,

it can be applied in molten form followed by solidification, or applied as solution in inorganic solvent and then freed of the solvent, or also applied as oil-in-water emulsion with subsequent drying (Kester and Fennema 1986).

82.4.1.1 Waxes. These substances are non polar lipids; they are insoluble in bulk water, have a high hydrophobicity and are soluble in organic solvents (Cha and Chinnan 2004). Coating frozen meats with waxes eliminated fresh meat low-grading caused by discoloration and reduced packaging labor (Cutter and Sumner 2002).

82.4.1.2 Glycerides. Monoglycerides are mainly used in edible films as emulsifiers; they stabilize the emulsion film and increase adhesion between the film components with different hydrophobicity (Cha and Chinnan 2004).

82.4.2 Polysaccharide Based Films

Originally, polysaccharides have been used in the food industry to provide hardness, crispness, thickening, adhesiveness, and compactness because of their gel forming ability (Cutter and Sumner 2002). However, several polysaccharides and their derivates (including alginate, pectin, carragenan, starch, hydrolyzed starch, cellulose derivatives, various plant microbial gums, and chitosan) can also be used to produce films and coatings (Kester and Fennema 1986; Gennadios and others 1997). Phan and others (2005) suggest that functionality varies within the polysaccharide material, as agar and cassava derived starch have better functional properties than normal and waxy rice starch films.

Polysaccharide films generally exhibit limited water vapor barrier ability due to their hydrophilic nature; but they can prevent water condensation and reduce microbial growth on the surfaces of poultry (Gennadios and others 1997; Cha and Chinnan 2004). Polysaccharide films are selectively permeable to CO_2 and O_2, they can increase shelf-life of foods, by preventing dehydration, oxidation, rancidity, and surface browning. For all these reasons polysaccharide films are used in Japan for packing processed meats, including ham and poultry (Cutter and Sumner 2002; Cha and Chinnan 2004).

82.4.2.1 Alginates. Alginates are salts of alginic acid extracted from brown seaweeds *Macrocystis pyrifera*. They are linear copolymers of D-manopyranosyluronic acid and L-guluropyranosyluronic acid units (Cha and Chinnan 2004). Alginate films are formed by crosslinking with a calcium salt or other di- or trivalent metal ions, or by exposition to pH 3 or less (Kester and Fennema 1986; Krochta and De Mulder-Johnston 1997; Cutter and Sumner 2002). Calcium is more effective in gelling alginates than magnesium, manganese, aluminum, ferrous, and ferric ions. However, calcium chloride used at concentrations greater than 5 M imparts bitter flavor to foods (Cutter and Sumner 2002).

Thus, alginate films are impervious to oils and fats, but they are poor moisture barriers and have high oxygen permeability coefficient (Krochta and De Mulder-Johnston 1997; Buonocore and others 2005). However, gelatinous alginate coatings have been applied to various meats such as beef and pork cuts, and poultry parts to reduce dehydration acting by sacrifice, as they lose water before food (Kester and Fennema 1986; Krochta and De Mulder-Johnston 1997). Moreover, alginates are good oxygen barriers and can provide protection against oxidation of food ingredients (Kester and Fennema 1986; Krochta and De Mulder-Johnston 1997). In addition, alginates can improve flavor, texture, and batter adhesion (Krochta and De Mulder-Johnston 1997). In this context,

Earle received a patent for the calcium alginate gel edible coating that can be used to prevent dehydration and color changes in raw fish, meat, and poultry during refrigerated storage (Cha and Chinnan 2004).

82.4.2.2 Carrageenan. Carrageenan is a water-soluble galactose polymer extracted from the Irish moss called *Chondrus crispus* and other species of red seaweeds. It is composed of a mixture from at least five polymers designated as ι-, κ-, λ-, μ-, and υ-carrageenan; but ι-, κ-, and λ-carrageenan have food applications. Gelation of ι- and κ-carrageenan occurs with both monovalent and divalent cations; whereas, λ-carrageenan is a nongelling and thickening agent (Gennadios and others 1997; Cha and Chinnan 2004). Carrageenan has been applied on poultry meat by Meyer and co-workers. They dipped chicken parts into 40 g/L aqueous solution of carrageenan at 64°C, which slightly increased poultry shelf-life at 2°C (Gennadios and others 1997). They also observed that the inclusion of an antibiotic was more effective to retard spoilage of poultry; however, antibiotics are no longer approved for use on poultry meat as a preservative (Gennadios and others 1997).

82.4.2.3 Agar. Agar is a gum derived from a variety of red seaweeds of *Rhodophyceae* class; this biopolymer is mainly composed of galactose polymers separable into two distinctive fractions known as agaran and agaropectin, which contain 5–10% sulfate esters. Agar forms strong reversible gels characterized by their melting point at about 85ºC, far above the initial gelation temperature (Gennadios and others 1997). Agar coatings added with antibiotics, such as soluble salts of chlortetracycline, neomycin, oxytetracycline, and polymyxin have been used to extend shelf-life of poultry parts stored at 2°C (Gennadios and others 1997; Cha and Chinnan 2004).

82.4.2.4 Starch. Starch, which is composed by amylose and amylopectin, is a material derived from cereal grains, potatoes, tapioca, banana, mango, and so on. Films obtained from this polysaccharide are odorless, tasteless, colorless, nontoxic, biologically absorbable, semipermeable to carbon dioxide, and resistant to the passage of oxygen. Films may be prepared by thermal gelation or by cold gelatinization with NaOH; although thermal gelation produces films with better barrier and mechanical properties (Romero-Bastida and others 2005). Starch presents physical characteristics very similar to plastic films and exhibit thermoplastic behavior, but films tend to recrystallize during long-term storage to the detriment of their functionality (Cutter and Sumner 2002; Cha and Chinnan 2004; Romero-Bastida and others 2005). Amylose is responsible for the film-forming capacity of starches and amylopectine is brittle and noncontinuous (Cha and Chinnan 2004). In 1960, hydroxypropylated amylose films were extruded and then they were used to wrap frozen meat, poultry, and fish, protecting them during frozen storage but dissolving during thawing and cooking (Cutter and Sumner 2002). Recently, Fama and others (2005), as well as, Veiga-Santos and others (2005) demonstrate that the mechanical properties and microstructure and color of starch films may be affected by pH modifications and inclusion of sorbate, xanthan gum, and other additives such as sucrose, soybean oil, sodium phosphate, and propylene glycol.

82.4.2.5 Chitosan. Chitosan is the mayor organic skeletal substance of invertebrates and the cell wall of fungi and green algae (Cutter and Sumner 2002). It is produced commercially by deacetilating chitin obtained from shellfish waste (Krochta and De Mulder-Johnston 1997). Chitosan films are clear, tough, and flexible, with good oxygen barrier

properties; it is produced by treating chitin with alkali and inhibits fungi and pathogens (Krochta and De Mulder-Johnston 1997; Cutter and Sumner 2002).

82.4.2.6 Cellulose and Cellulose Derivates. Cellulose is the most abundant natural polymer on earth, and it is essentially a lineal natural polymer of anhydroglucose. Due to its chemical structure, it is highly fibrous, and insoluble (Cha and Chinnan 2004). Cellulose derivates such as methylcellulose (MC), hydroxipropyl cellulose (HPC), hydroxipropyl methylcellulose (HPMC), and carboxymethylcellulose (CMC) are water-soluble ethers with good film forming properties (Gennadios and others 1997). They produce films resistant to fats and oils, tough, and flexible (Cutter and Sumner 2002). They may be combined with oil as a plasticizer to coat both fresh and frozen meats (Cutter and Sumner 2002). Edible films made from MC and HPMC have been useful to reduce the quantity of fat and to reduce moisture loss of meat balls prepared from ground chicken breast (Gennadios and others 1997; Holownia and others 2000). Holownia and others (2000) also observed that chicken strips coated with MC and HPMC hindered the migration of moisture and acetic acid into the frying oil and this activity was responsible for reduced free fatty acid generation in those oils used to fry the coated products.

82.4.3 Protein Based Films

Proteins commanded great attention as degradable, renewable polymer films (Petersen and others 1999). They can be manufactured from animal and vegetable resources such as collagen, gelatin, milk proteins, wheat gluten, soy proteins, rice, corn zein, peanut, egg albumen, and so on (Gennadios and others 1997; Cutter and Sumner 2002). Protein film formation results from polymerization of denatured protein chains, where disulfide, hydrogen, and hydrophobic interactions form a continuous film network; thus proteins containing high SH-aminoacid residues as cysteine have better film properties (Were and others 1999). Protein based-films have better mechanical and barrier properties than polysaccharides (Khwaldia and others 2004). These films adhere well to hydrophilic surfaces and provide good barriers for oxygen and carbon dioxide, but do not resist water diffusion (Cutter and Sumner 2002). Their limited resistance to water vapor transmission is attributed to the hydrophilic protein nature, as well as to the presence of hydrophilic plasticizers, such as glycerin and sorbitol, incorporated to impart flexibility (Gennadios and others 1997). Protein-based packaging presents some disadvantages, for example, they may be degraded by the endogenous enzymes present in raw meats; additionally, they may induce health problems, especially in people with food allergies to milk, egg, peanut, soybean, or gluten proteins (Gennadios and others 1997; Cutter and Sumner 2002).

82.4.3.1 Gelatin. Gelatin derivates from the partial hydrolysis of collagen. Gelatin coatings have been used as carriers directly into processed poultry meat surfaces to prevent microbial growth, grease bleeding, handling abuse, water transfer, moisture loss, and oil adsorption during frying. The preservation of meat and other foods with gelatin coatings was proposed in 1869 (Gennadios and others 1997; Cutter and Sumner 2002).

82.4.3.2 Corn Zein. Corn zein is the prolamine fraction or corn protein (Cha and Chinnan 2004), which give rise to strong water insoluble films, resistant to microbial attack (Weller and others 1998; Fu and others 1999; Padua and Wang 2002). Corn zein films have been commercially used in coating formulations for shelled nuts, candy, and

pharmaceutical tablets. They provide a good barrier to oxygen, and its water vapor permeability is about 800 times higher than that of a typical shrink-wrap film (Cha and Chinnan 2002).

The use of corn zein was suggested for poultry preservation by Herald and others (1996). They wrapped turkey breast with corn zein film added with antioxidant (butylated hidroxyanisole) and emulsifier (acetylated monoglyceride) and observed lower hexanal content in these samples in comparison with samples packaged in PVDC. Janes and others (2002) demonstrated that zein coating films added with nisin can prevent the growth of *Listeria monocytogenes* on ready-to-eat chicken. Also, Lungu and Johnson (2005a,b) incorporated nisin, sodium diacetate, and sodium lactate to inhibit *Listeria monocytogenes* on turkey frankfurters.

82.4.3.3 Soy Protein Isolates. Soy bean proteins are composed by two major componets, 11S (glycinin) and 7S (β-conglycinin); the 11S fraction has better film forming properties than the 7S (Yildirim and Hettiarachchy 1997). However, the film forming ability of soy protein has traditionally been utilized in the Far East for the production of soy protein-lipid called *yuba* films. Soy based films with added lauric acid and nisin have been used successfully to reduce the growth of *Listeria monocytogenes* on turkey Bologna (Dawson and others 2002).

82.4.3.4 Whey Protein Isolate. The use of milk proteins to produce films and coatings and their characteristics has been analyzed by Khwaldia and others (2004). Whey protein isolate produces transparent, flexible, edible films with excellent oxygen, and aroma barrier properties at relatively low humidity. These films are water insoluble, however, due their hydrophilic nature they are less effective moisture barriers. Whey protein coatings have been used to improve food integrity and reduce loss due damage in freeze-dried chicken (Pérez-Gago and Krochta 1999, 2002).

82.4.4 Composite Films

Edible packaging made from proteins, lipids, and polysaccharides or by their mixture presents distinct functional characteristics (Wu and others 2002). Each film material has its own particular functionality and application according to its molecular structure. Pure lipid films have extremely low water vapor permeability because their hydrophobic nature, but they are opaque and relatively inflexible; while protein and polysaccharide films exhibit low gas permeability and poor water barrier properties with suitable mechanical and optical properties (Kester and Fennema 1986; Guilbert and others 1996; Wu and others 2002). However, when lipids are incorporated into protein and/or polysaccharide films, the water permeability is reduced by either laminating a hydrophilic lipid layer or making a composite film where both the hydrophobic and hydrophilic components and dispersed (Ko and others 2001). For instance Chung and Lai (2005) reported that starch-beeswax composite films had better water-barrier properties than those simple starch films by using magnetic resonance. In addition it seems that protein-lipid composite films have better moisture barrier properties than those made of polysaccharides, as reported by Ryu and others (2005) for zein and high amylose corn starch composite films mixed with polyethylene glycol, glycerol, and oleic acid.

82.5 MECHANISMS TO MANUFACTURE EDIBLE FILMS AND COATINGS

Selection of edible materials for films and coatings for poultry meat and meat products, the nature of the materials, the manufacturing process and the application method on foods, as well as moisture barrier ability, water and lipid solubility, color, appearance, mechanical, rheological characteristics, manufacturing method, and toxicity need to be considered before selecting an edible packaging material (Cutter and Sumner 2002). These properties depend on the type of material used, its formation and application. Plasticizers, crosslinking agents, antimicrobials, antioxidants, texture agents, and so on, can be added to enhance functional properties of films (Guilbert and others 1996).

The mechanisms for film and coating formation have been analyzed by several authors (Kester and Fennema 1986; Guilbert and others 1996; Khwaldia and others 2004). According to Guilbert and others (1996) edible films and coatings can be formed by simple coacervation, complex coacervation and gelation or thermal coagulation.

- Simple coacervation: where a hydrocolloid dispersed in water is separated from the solvent phase due to its compatibility. This process takes place after solvent evaporation, previous addition of a hydrosoluble nonelectrolyte, in which hydrocolloid is not soluble, of after pH adjustment.
- Complex coacervation: where two hydrocolloid solutions with opposite electron charges are mixed, causing electrostatic interactions and precipitation of the polymer complex (Khwaldia and others 2004).
- Gelation or thermal coagulation: where heating of the hydrocolloid, which leads to its denaturalization followed by gelation or precipitation; also cooling polymer dispersion may cause gelation. However, hot casted films show lower gas diffusion than cool-casting films (Lukasik and Ludescher 2006). In particular, globular protein films are produced by thermal gelation followed by dehydration.

82.6 SUBSTANCES USED TO IMPROVE MECHANICAL PROPERTIES OF FILMS

In order to improve functional characteristics from films, addition of other substances such as plasticizers, crosslinking agents, and lipids are incorporated into film forming solutions (Cutter and Sumner 2002). Plasticizers are compounds of low volatility which may be added to impart flexibility to a polymer film (examples of these are glycerol, propylene glycol, sorbitol, manitol, sucrose, polyethylene glycol, etc.) by weakening intermolecular forces between adjacent polymer chains, thus decreasing tensile strength while the film flexibility increases (Kester and Fennema 1986; Krochta 2002). Unfortunately, plasticizers also decrease the barrier properties against moisture, oxygen, aroma, and oils (Krochta 2002). The heat applied during protein thermal gelation induces aggregation and conversion of the regular secondary structures into antiparallel beta-sheets, while dehydration induces further aggregation. The presence of the plasticizer allows specific conformational rearrangements into extended beta-sheets and ordering of the polypeptide chains improving film functionality (Lefevre and others 2005). Tang and others (2005) have suggested that moisture barrier properties are highly correlated with the surface hydrophobicity film network. The incorporation of microbial transglutaminase in combination with various

plasticizers for film soy protein isolates can slow down the moisture loss rate during the drying process due to the increase of surface hydrophobicity. This increase generates a more homogeneous or compact films structure compared to the controls. Transglutaminase enhances globular protein crosslinking by introducing ε-(γ-Glu)-Lys intra and intermolecular covalent bonds that improve film water resistance (Yildirim and Hettiarachchy 1997). Other crosslinking agents include formaldehyde, glutaraldehyde, and glyoxal that impart greater mechanical strength to gliadin films (Hernandez-Muñoz and others 2005). Moreover, incorporation of waxes and/or fatty acids (such as beeswax, estearic and palmitic acids, among others) improves moisture barrier properties; however, the barrier efficiency will depend on the polarity of components and on the homogenous dispersion of hydrophobic substances (Pérez-Gago and Krochta 1999; Yang and Paulson 2000).

82.7 METHODS FOR APPLICATION OF EDIBLE FILMS AND COATINGS

Edible coatings are applied and/or formed directly on the food product surface; whereas edible films are structures that are applied after being previously formed (Guilbert and others 1996). Edible films can be applied on foods by wrapping, dipping, brushing, spraying, casting, and foaming (Cutter and Sumner 2002; Wu and others 2002). Selection of the method will depend on the food type and cost. Additionally, shelf-supporting films can be obtained by techniques such as extrusion, molding, or rolling mill procedures (Guilbert and others 1996).

82.8 SAFETY AND HEALTH CONSIDERATIONS OF EDIBLE FILMS

One of the most important topics in the production of edible films is the selection of ingredients because all of them have to be safe. According to Krochta and De Mulder-Johnston (1997), there are several safe aspects that have to be studied before film formulation:

1. Edible film polymers have to be generally recognized as safe (GRAS), this joined to good manufacturing practices.
2. If the edible ingredient is not currently GRAS but the manufacture can demonstrate safety, the manufacturer can apply for a GRAS petition to the Food Drug Administration (FDA) or proceed to mark the material without FDA concurrence (shelf-determination).
3. Manufacturer may not need to establish that use of the edible polymer in edible films is GRAS if the material received pre-1958FDA clearance and thus has "prior sanction."
4. If the material cannot be demonstrated to be GRAS or "prior to sanction," the manufacturer must submit a food additive petition of FDA.

There are edible polymers used for packaging manufacture (films or coatings) that have received special attention. They are: cellulose ethers, starch, hydroxypropilated starch, corn zein, wheat gluten, soy protein, and milk proteins because some people have wheat gluten intolerance, milk protein allergies, or lactose intolerance (Krochta and De Mulder-Johnston 1997). Hence, it is necessary to declare when these materials have been used in the manufacture of edible packaging (Krochta and De Mulder-Johnston 1997).

82.9 ACTIVE AND EDIBLE PACKAGING

Nowadays, there are big efforts for innovation of food packaging technology. These efforts are result of the increasing demand for minimally processed foods and the change in retail and distribution practices associated with the globalization such as new consumer products logistics, new distribution tendencies, and automatic handling system and distribution centers. These factors have contributed to the development of a new concept of packaging known as active packaging (Suppakul and others 2003; Yam and others 2005). An active packaging is a mode of packaging in which the package, the product, and the environment interact to prolong shelf-life or enhance safety or sensory properties while maintaining the quality of the product (Suppakul and others 2003). In general, active food packaging can provide several functions that do not exist in conventional packaging systems (Quintavalla and Vicini 2002). These functions include: oxygen scavenging systems, moisture-absorbing control systems; carbon dioxide generating systems, ethanol generating systems, antioxidant systems, and antimicrobial migrating/not migrating systems (Suppakul and others 2003).

Oxygen scavenger systems remove the residual oxygen after packaging. This technology is based on oxidation of iron power, ascorbic acid, photo sensitive dyes, enzymes (glucose oxidase), unsaturated fatty acids (such as oleic, linoleic, and linolenic acids), rice extract or immobilized yeast on a solid subtract (Suppakul and others 2003). This kind of packaging can be used to prevent the growth of aerobic bacteria and molds (Han 2000a,b). They are also useful for products that are sensitive to oxygen and light (Suppakul and others 2003). While systems of water absorption used to control liquid water in foods consist in a super absorbent polymer (such as polyacrilate salt or starch graft copolymers) located between two layers of a micro-porous polymer (Suppakul and others 2003).

82.9.1 Carbon Dioxide Generating Systems

Carbon dioxide generating systems are useful in certain applications such as fresh meat, poultry, fish, and cheese packaging (Suppakul and others 2003).

82.9.2 Ethanol Generating Systems

Ethanol prevents microbial spoilage of intermediate moisture foods such as cheeses and bakery products. In addition, it can reduce the rate of staling and oxidative changes (Suppakul and others 2003).

82.9.3 Antioxidants

One of the main problems for raw and cooked poultry meat quality is to prevent lipid oxidation. Traditionally, antioxidants of synthetic origin such as butyl hydroxyanisol (BHA) or butyl hydroxytoluene (BHT) and terbutyl hydroquinine (TBHQ) have been used delay the onset of oxidative rancidity in meat products by sequestering free radicals. However, in recent years there has been increasing interest in the use of natural antioxidants due to consumer concerns about the potential toxicity of some synthetic antioxidants. Natural antioxidants such as α-tocopherol, rosemary (*Rosmarinus officinalis* L.), or sage (*Buddleia perfoliata* Kunth), basil, and garlic extracts, among other

spices and herbal extract may be used to delay rancidity (Gruen 2005). Armitage and others (2002) incorporate fenugreek seeds, rosemary and vitamin E as antioxidants in egg albumen films to reduce lipid oxidation in cooked and uncooked poultry; but no differences were detected in poultry samples coated with egg albumen containing antioxidants, data suggested that the use of egg albumen coating was responsible for lipid oxidation delay.

82.10 ANTIMICROBIAL FOOD PACKAGING

Antimicrobial packaging is a promising area in active packaging for meat poultry products (Quintavalla and Vicini 2002). The interest in this packaging has increased in recent years due to a concern over the risk of food borne illness, and advances in the film production technology (Dawson and others 2002). One example of antimicrobial food packaging is the antimicrobial absorbent foods pads used in trays for packed retail meats and poultry to soak up meat juice. This pad has the capacity to inhibit food borne pathogens due the presence of anionic surfactants such as alkyl sulfonate salts, alkyl sulfate salts, as well as citric and malic acids (Cha and Chinan 2004; Brody 2005).

Antimicrobial food packaging allows extending the lag period and thus reducing the growth of microorganisms, improving shelf-life and food safety (Han 2000a; Suppakul and others 2003). Active packaging can be classified in two groups: (1) those were the antimicrobial agent migrates to the surface of the food, and (2) those that are effective against surface growth microorganisms without migration (Suppakul and others 2003; Cha and Chinnan 2004). Although, antimicrobial films may also incorporate radiation-emitting materials (Cha and Chinnan 2004; Brody 2005).

Several compounds are used as antimicrobials; these include: organic acids, enzymes, lisozyme, EDTA, essential oils, chitosan, lactoperoxidase system, lactoferrine, and bacteriocins (Ouattara and others 2000; Cha and Chinan 2004; Min and Krochta 2005; Min and others 2005; Quintero-Salazar and others 2005).

Most works related to antimicrobial packaging have focused their attention on the application of bacteriocins, described peptides with antibacterial inhibitory action produced by some lactic acid bacteria; as antimicrobial agents into edible and nonedible films (see Fig. 82.1 as well as Tables 82.1 and 82.2). One of the first reports was made by Cutter and Siragusa (1996, 1997) for films made of alginates added with nisin to inhibit

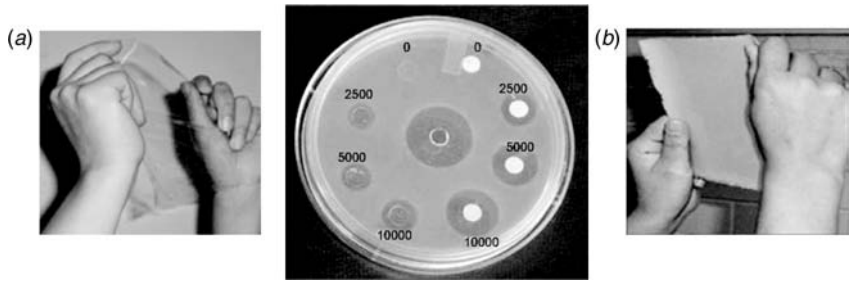

Figure 82.1 *Inhibition halos generated by (a) transparent whey protein isolate films, and (b) opaque corn zein films (added with several levels of the bacteriocin pediocin from 0 up to 10000 IU/mL of film forming solution) placed on an agar plate inoculated with Listeria innocua ATCC 33090 incubated at 37°C for 18 h.*

TABLE 82.1 Application of Several Antimicrobial Agents on Different Systems of Edible Forming Packaging.

Film Material	Antimicrobial	Target Microorganism	Reference
		Proteins	
Whey protein isolate	Lactoferrine Lactoperoxidase	*Penicillium commune*	Min and Krochta 2005
	Nisin	*Listeria monocytogenes*	Ko and others 2001
	Pediocin	*Listeria innocua*	Quintero-Salazar and others (2005)
	p-Aminobenzoic acid Sorbic acid	*Listeria monocytogenes* *Salmonella tiphymurium* *Escherichia coli O157:H7*	Cagri and others 2001
Zeín	EDTA, lauric acid Nisin	*Listeria monocytogenes* *Salmonella enteritis*	Hoffman and others 2001
	Pediocin	*Listeria innocua*	Quintero-Salazar and others 2005
Soy protein isolate	Lisozyme	*Lactobacillus platarum*	Padgett and others 1998
Zeín	Nisin EDTA	*E. coli*	
	Citric acid	*Listeria monocytogenes*	Eswaranandam and others 2004
	Lactic acid Tartaric acid Nisin	*Escherichia coli O157:H7* *Salmonella graminaria*	
		Polysaccharides	
Na-alginate	Losozyme, nisin, grape seeds extract, EDTA	*Micrococcus luteus*	Cha and others 2003
K-carragenin		*Listeria innocua* *Salmonella enteritis* *Escherichia coli* *Staphylococcus aureus*	
Chitosan	Garlic oil, potassium sorbate, nisin	*Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Listeria monocytogenes*	Pranoto and others 2005
Hydroxypropyl metyl-celulosae (HMPC)	Nisin	*Micrococcus luteus*	Sebti and others 2003
	Nisin	*Listeria innocua* *Staphylococcus aureus*	Sebti and Coma 2002
HMPC	Nisin	*Listeria innocua* *Staphylococcus aureus*	Coma and others 2001
HMPC	Nisin	*Listeria monocytogenes* *Staphylococcus aureus*	Sebti and others 2002

Brochotrix thermosphacta. These authors suggested that nisin was more effective when it was immobilized into alginate gels that when it was added directly to muscle substrates. Also, Ming and others (1997) incorporated the bateriocins, nisin, and pediocin into cellulose films, to completely inhibit *Listeria monocytogenes* growth on ham, chicken, and beef

TABLE 82.2 Examples of Incorporation of Antimicrobial Substances Films and Coatings and Their Application on Foods.

Biopolymer	Antimicrobial	Target Microorganism	Food System	Reference
		Proteins		
Whey protein isolate (WPI)	Lactoperoxidase system	*Listeria monocytogenes*	Smoked salmon	Min and others 2005
	p-Aminobenzoic acid	*Listeria monocytogenes*	Hot dogs	Cagri and others 2003
	p-Aminobenzoic acid	*Listeria monocytogenes*	Bologna and sausages	Cagri and others 2001
	Sorbic acid	*E. coli* O157:H7 *Salmonella tiphymurium*		
WPI/calcium caseinate	Sorbic acid	*Enterobacteriaceae* *Pseudomona*	Ground meat	Ouattara and others 2002
Soy protein isolate	Nisin	*Listeria monocytogenes*	Turkey bologna	Dawson and others 2002
	Lauric acid			
	Nisin	*Listeria monocytogenes*	Turkey frankfurters	Theivendran and others 2006
	Grape seed extract			
Zeín	Nisin	*Listeria monocytogenes*	Turkey Sausages	Lungu and Johnson 2005a,b
	Potassium sorbate			
	Sodium diacetate			
	Sodium lactate			
	Nisin	*Listeria monocytogenes*	Chicken	Janes and others 2002
		Polysaccharides		
Calcium alginate	Nisin	*Salmonella tiphymurium*	Chicken	Natrajan and Sheldon 2000b
	Citric acid			
	EDTA			
	Nisin	*Brochotrix thermosphacta*	Ground meat	Cutter and Siragusa 1997
	Trisodium phosphate and sodium chloride	*Listeria innocua*	Chicken carcass	Mehyar and others 2005
Chitosan	Acetic acid	*Enterobacteriaceae*	Meat products	Ouattara and others 2000
	Propionic acid	*Serratia liquefaciens*		
Metylcelullose	Nisin	*L. monocytogenes*	Tofu	Cha and others 2003
	Nisin/lacticin 3147	*Lactococcus lactis*	Cheese	Scannell and others 2000
		L. innocua *Staphilococcus aureus*	Ham	
Cellulose	Nisin	*L. monocytogenes*	Ham	Ming and others 1997
	Pediocin		Beef meat	
			Turkey breast	

meat, during 12 weeks. In 1998, Padgett and others demonstrated that the inhibitory inhibition spectrum of corn zein and soy protein edible films added with nisin could be increased against Gram-negative bacteria when nisin was added in combination with lisozyme and EDTA. In 1999 Sigarusa and others, as well as, Cutter and others (2001)

observed that nisin antimicrobial activity was maintained in a synthetic polymer of nylon and it was useful in to inhibit the growth of *Lactobacillus helveticus* and *Brochotrix thermosphacta* when applied on beef carcasses. Scannell and others (2000) incorporated nisin and lacticin 3147 into a polyethylene/polyamide polymer and cellulose to inhibit *Listeria innocua* and *Staphylococcus aureus* in cheese and ham. Natrajan and Sheldon (2000a) incorporated nisin, EDTA, citric acid, and tween 80 into synthetic polymers and into absorbent pads to inhibit the growth of *Salmonella thypphymurium* on chicken skin. The same authors also used protein and polysaccharide-based films added with nisin to inhibit *Salmonella* on poultry skin (Natrajan and Sheldon 2000b). Ouattara and others (2000) prepared antimicrobial films by incorporating acetic or propionic acid into chitosan matrix, with or without lauric acid or cinnamaldehyde to be applied onto bologna, regular cooked ham. Hoffman and others (2001) incorporated EDTA, lauric acid and nisin into corn zein which against *Listeria monocytogenes* and *Salmonella enteritis*. Dawson and others (2002) used soy protein films added with nisin and lauric acid to inhibit *Listeria monocytogenes* in Bologna turkey. While, Cha and others (2003), made films of sodium aginate and carragenan added with EDTA, lisozyme, nisin, and grape extract against *Micrococcus luteus. Listeria innocua*, *Salmonella enteritis*, *Escherichia coli*, and *Staphylococcus aureus*. Eswaranandam and others (2004) incorporated organic acids and nisin into soy films to inhibit *Listeria monocytogenes* and *E. coli* O157:H7. Lungu and Johnson (2005a,b) established that treatments using nisin alone or in combination with zein, etanol-glycerol, or propylene glycol was appropriated to be applied on ready-to-eat foods, promising for use the use of antimicrobial films as barriers against L. *monocytogenes* recontamination on turkey frankfurters.

Most work using antimicrobial edible films have been done on model poultry meat products; however, there is a need to explore industrial applications to ensure safety as part of the hurdle technology for poultry preservation.

82.11 CONCLUSIONS

Edible packaging has been applied over several years on a number of foods including fresh, frozen, and processed poultry products, as a means of preservation to improve quality and safety. There are many opportunities for research in this area in order to improve mechanical and barrier properties, or develop new packaging concepts according to the consumer demands and processors' needs in order to renew the packaging technology, using natural and biodegradable under-valued materials from animal and vegetable resources; as well as by the inclusion of additives to improve mechanical, barrier and other functional characteristics to ensure poultry quality.

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Section XVI

Seafood

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83

Frozen Seafood Products Description

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83.1 Introduction 822
83.2 Frozen Headless Dressed Whiting 822
83.2.1 Product Description 822
83.2.2 Grades of Frozen Headless Dressed Whiting 822
83.2.3 Determination of the Grade 822
83.3 Frozen Halibut Steaks 823
83.3.1 Product Description 823
83.3.2 Styles of Frozen Halibut Steaks 823
83.3.3 Recommended Dimensions 823
83.3.4 Color Defects 824
83.4 Frozen Salmon Steaks 824
83.4.1 Product Description 824
83.4.2 Species 824
83.4.3 Styles 825
83.4.4 Recommended Dimensions 825
83.4.5 Color Defects 825
83.5 Frozen Fish Fillet Blocks 826
83.5.1 Product Description 826
83.5.2 Definitions of Defects 826
83.5.2.1 Dehydration 826
83.5.2.2 Uniformity of Block Size 826
83.5.2.3 Underweight 826
83.5.2.4 Angles 826
83.5.2.5 Improper Fill 827
83.5.2.6 Belly Flaps (Napes) 827

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

83.5.2.7 Blood Spots 827
83.5.2.8 Bruises 827
83.5.2.9 Discoloration 827
83.5.2.10 Viscera, Roe, and Lace 827
83.5.2.11 Skin 827
83.5.2.12 Membrane (Black Belly Lining) 828
83.5.2.13 Scales 828
83.5.2.14 Foreign Material 828
83.5.2.15 Bones 828
83.5.2.16 Fins or Part Fins 828
83.5.2.17 Parasites 828
83.5.2.18 Texture 828
83.6 Frozen Minced Fish Blocks 829
83.6.1 Product Description 829
83.6.2 Product Forms 829
83.6.2.1 Types 829
83.6.2.2 Color Classifications 829
83.6.2.3 Texture 829
83.6.3 Definitions of Defects 829
83.6.3.1 Deteriorative Color 829
83.6.3.2 Dehydration 830
83.6.3.3 Uniformity of Size 830
83.6.3.4 Uniformity of Weight 830
83.6.3.5 Angles 830
83.6.3.6 Improper Fill 830
83.6.3.7 Blemishes 831
83.6.3.8 Bones 831
83.6.3.9 Flavor and Odor 831
83.6.3.10 Texture Defects 831
83.6.4 Additives 831
83.6.5 Hygiene 832
83.7 Frozen Raw Fish Portions 832
83.7.1 Product Description 832
83.7.2 Styles 832
83.7.3 Types 832
83.7.4 Definitions of Defects 832
83.7.4.1 Dehydration 832
83.7.4.2 Uniformity of Size 832
83.7.4.3 Uniformity of Weight 832
83.7.4.4 Blemishes 832
83.7.4.5 Bones 833
83.7.4.6 Texture Defects 833
83.7.5 General Definitions 833
83.8 Frozen Raw Breaded Fish Sticks 833
83.8.1 Product Description 833
83.8.2 Composition of the Product 834
83.8.3 Definitions 834
83.8.3.1 Examination of Sample, Frozen State 834
83.8.3.2 Examination of Sample, Cooked State 835
83.9 Frozen Raw Breaded Fish Portions 835
83.9.1 Product Description 835

83.9.2 Styles 836
83.9.3 Composition of the Product 836
83.9.4 Examination of Sample, Frozen State 836
83.9.5 Examination of Sample, Cooked State 836
83.10 Frozen Fried Fish Sticks 837
83.10.1 Product Description 837
83.10.2 Composition of the Product 838
83.10.3 Definitions of Factors for Point Deductions 838
83.10.3.1 Examination of Sample, Frozen State 838
83.10.3.2 Examination of Sample, Cooked State 838
83.11 Frozen Fried Fish Portions 839
83.11.1 Product Description 839
83.11.2 Composition of the Product 839
83.11.3 Examination of Sample, Frozen State 840
83.12 Fresh And Frozen Shrimp 840
83.12.1 Product Description 840
83.12.2 Types 840
83.12.3 Styles 840
83.12.4 Market Forms 841
83.12.5 Examination of Sample, Frozen State 841
83.12.6 Examination, Sample, Fresh or Thawed State 841
83.12.7 Examination of Sample, Cooked State 842
83.13 Frozen Raw Breaded Shrimp 843
83.13.1 Product Description 843
83.13.2 Styles 843
83.13.3 Types 844
83.13.4 Definitions and Methods of Analysis 844
83.13.4.1 Fantail Shrimp 844
83.13.4.2 Round Shrimp 844
83.13.4.3 Composite Units 844
83.13.5 Factors Evaluated on Unbreaded or Thawed Debreaded Product 845
83.14 Frozen Raw Scallops 846
83.14.1 Product Description 846
83.14.2 Styles 846
83.14.3 Types 847
83.14.4 Definitions 847
83.15 Frozen Raw Breaded Scallops and Frozen Fried Scallops 847
83.15.1 Product Description 847
83.15.1.1 Frozen Raw Breaded Scallops 847
83.15.1.2 Frozen Fried Scallops 847
83.15.2 Styles 848
83.15.3 Types 848
83.15.4 Definitions 848
83.15.5 Workmanship Defects 848
83.15.6 Texture in the Cooked State 849
83.15.6.1 Texture of the Coating 849
83.15.6.2 Texture of the Scallop Meat 849
83.15.7 Character 849
83.16 North American Freshwater Catfish and Catfish Products 849
83.16.1 Scope and Product Description 849
83.16.2 Product Presentation 850

83.16.2.1 Types 850
83.16.2.2 Styles 850
83.16.2.3 Market Forms 850
83.16.2.4 Bone Classifications 850
83.16.3 Definitions 850

83.1 INTRODUCTION

This book is not the proper forum to discuss the manufacture of every frozen seafood product available in the market. However, regulatory agencies such the National Marine Fisheries Service (NMFS) have issued some minimal criteria for several frozen seafood and seafood products: what they are, what types and styles are available, and so on. The information in this chapter describes each available frozen seafood product and has been modified from the product grades issued by the NMFS. A product grade is established to achieve two objectives: to assure product safety and to minimize economic fraud.

83.2 FROZEN HEADLESS DRESSED WHITING

83.2.1 Product Description

The product described in this part consists of clean, wholesome whiting (silver hake) *Merluccius bilineraris*, *Merluccius albidus* completely and cleanly headed and adequately eviscerated. The fish are packaged and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product.

83.2.2 Grades of Frozen Headless Dressed Whiting

U.S. Grade A is the quality of frozen headless dressed whiting that possess a good flavor and odor.

U.S. Grade B is the quality of frozen headless dressed whiting that possess at least reasonably good flavor and odor.

Substandard or Utility is the quality of frozen headless dressed whiting that otherwise fail to meet the requirements of U.S. Grade B.

83.2.3 Determination of the Grade

Good flavor and odor (essential requirements for a U.S. Grade A product) means that the cooked product has the typical flavor and odor of the species and is free from rancidity, bitterness, staleness, and off-flavors and off-odors of any kind.

Reasonably good flavor and odor (minimum requirements of a U.S. Grade B product) means that the cooked product is lacking in good flavor and odor but is free from objectionable off-flavors and off-odors of any kind.

Arrangement of product refers to the packing of the product in a symmetrical manner, bellies or backs all facing in the same direction, fish neatly dovetailed.

Condition of the packaging material refers to the condition of the cardboard or other packaging material of the primary container.

If the fish is allowed to stand after packing and prior to freezing, moisture from the fish will soak into the packaging material and cause deterioration of that material.

Dehydration refers to the presence of dehydrated (water-removed) tissue on the exposed surfaces of the whiting. Slight dehydration is surface dehydration that is not color-masking. Deep dehydration is color-masking and cannot be removed by scraping with a fingernail.

Minimum size refers to the size of the individual fish in the sample. Fish that are two ounces or over are considered acceptable. Smaller fish cannot be cooked uniformly with acceptable size fish.

Heading refers to the condition of the fish after they have been headed. The fish should be cleanly headed behind the gills and pectoral fins. No gills, gill bones, or pectoral fins should remain after the fish have been headed.

Evisceration refers to the cleaning of the belly cavities of the fish. All spawn, viscera, and belly strings should be removed.

Scaling refers to the satisfactory removal of scales from the fish.

Color of the cut surfaces refers to the color of the cut surfaces of the fish after heading and other processing.

Bruises and broken or split skin refers to bruises over one-half square inch in area and splits or breaks in the skin more than one-half inch in length that are not part of the processing.

Texture defects refers to the absence of normal textural properties of the cooked fish flesh, which are tenderness, firmness, and moistness without excess water. Texture defects are dryness, softness, toughness, and rubberyness.

83.3 FROZEN HALIBUT STEAKS

83.3.1 Product Description

Frozen halibut steaks should be clean, wholesome units of frozen raw fish flesh with normally associated skin and bone and should be two ounces or more in weight. Each steak should have two parallel surfaces and be derived from whole or subdivided halibut slices of uniform thickness that result from sawing or cutting perpendicular to the axial length, or backbone, of a whole halibut. The steaks, prepared from either frozen or unfrozen halibut (*Hippoglossus* spp.), should be processed and frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

83.3.2 Styles of Frozen Halibut Steaks

Style I: Random weight pack. The individual steaks are of random weight and neither the weight nor the range of weights are specified.

Style II: Uniform weight or portion pack. All steaks in the package or in the lot are of a specified weight or range of weights.

83.3.3 Recommended Dimensions

1. The recommended dimensions of frozen halibut steaks are not incorporated in the grades of the finished product since dimensions, as such, are not factors of quality for the purpose of these grades. However, the degree of uniformity of thickness among units of the finished product is rated since it is a factor affecting the quality and utility of the product.
2. It is recommended that the thickness (smallest dimension) of individually frozen halibut steaks be not less than $\frac{1}{2}$ inch and not greater than $1\frac{1}{4}$ inches.

Percentage glaze on halibut steak means the percent by weight of frozen coating adhering to the steak surfaces and includes the frost within the package.

Uniformity of thickness means that the thickness is substantially the same for one or more steaks within a package or sample unit.

83.3.4 Color Defects

1. Discoloration of drip liquor means that the free liquid that drains from the thawed steaks is discolored with blood residue usually from the dorsal aorta of the halibut.
2. Discoloration of light meat means that the normal flesh color of the main part of the halibut steak has darkened due to deteriorative influences.
3. Discoloration of the dark meat means that the normal color of the surface fat shows increasing degrees of yellowing due to oxidation.
4. Non-uniformity of color refers to noticeable differences in color on a single steak or between adjacent steaks in the same package.
5. Dehydration refers to the appearance of a whitish area on the surface of a steak due to the removal of water or drying of the affected area.
6. Honeycombing refers to the visible appearance of numerous discrete holes or openings of varying size on the steak surface.
7. Workmanship defects refers to appearance defects that were not eliminated during processing and are considered either objectionable or poor commercial practice.
8. Texture defect refers to an undesirable increase in toughness and/or dryness, fibrousness, and watery nature of halibut examined in the cooked state.

83.4 FROZEN SALMON STEAKS

83.4.1 Product Description

Frozen salmon steaks should be clean, wholesome units of frozen raw fish flesh with normally associated skin and bone and should be $2\frac{1}{2}$ ounces or more in weight. Each steak should have two parallel surfaces and be derived from whole or subdivided salmon slices of uniform thickness that result from sawing or cutting dressed salmon perpendicularly to the axial length, or backbone. The steaks, prepared from either frozen or unfrozen salmon (*Oncorhynchus* spp.), should be processed and frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product. The steaks in an individual package should be prepared from only one species of salmon.

83.4.2 Species

Frozen salmon steaks covered by this standard are prepared from salmon of any of the following species:

Silver or Coho (*O. kisutch*).

Chum or Keta (*O. keta*).

King, Chinook, or Spring (*O. tshawytscha*).

Red, Sockeye (*O. nerka*).
Pink (*O. gorbuscha*).

83.4.3 Styles

Style I: Random weight pack. The individual steaks are of random weight and neither the individual steak weight nor the range of weights is specified. The steaks in the lot represent the random distribution cut from the head to tail of a whole dressed salmon.

Style II: Random weight combination pack. The individual steaks are of random weight and neither the individual steak weight nor range of weights is specified. The steaks in the lot represent a combination of cuts from selected parts of the whole dressed salmon.

Style III: Uniform weight or portion pack. All steaks in the package or in the lot are of a specified weight or range of weights.

83.4.4 Recommended Dimensions

It is recommended that the thickness (smallest dimension) of individually frozen salmon steaks be not less than $\frac{1}{2}$ inch and not greater than $1\frac{1}{2}$ inches.

General appearance defects refer to poor arrangement of steaks, distortion of steaks, wide variation in shape between steaks, greater than normal number of head and/or tail pieces, imbedding of packaging material into fish flesh, inside condition of packaging, frost deposit, excessive or non-uniform skin glaze, and undesirable level of natural color.

Dehydration refers to the appearance of a whitish area on the surface of a steak due to the evaporation of water or drying of the affected area.

Uniformity of thickness means that the steak thickness is within the allowed manufacturing tolerance between the thickest and thinnest parts of the steaks within a package or sample unit.

Workmanship defects refers to appearance defects that were not eliminated during processing and are considered objectionable or poor commercial practice. They include the following: Blood spots, bruises, cleaning (i.e., inadequate cleaning of the visceral cavity of blood, viscera and loose or attached appendages), cutting (i.e., irregular, inadequate, unnecessary, or improper cuts and/or trimmings), fins, foreign material (i.e., any loose parts, of fish or other than fish origin), collar bone, girdle (i.e., bony structure adjacent to fin), loose skin, pugh marks, sawdust and scales.

83.4.5 Color Defects

Discoloration of fat portion means that the normal color of the fat shows increasing degrees of yellowing due to oxidation.

Discoloration of lean portion means that the normal surface flesh color has faded or changed due to deteriorative influences.

Nonuniformity of color refers to noticeable differences in surface flesh color on a single steak or between adjacent steaks in the same package or sample unit. It also includes color variation of the visceral cavity and skin watermarking.

Honeycombing refers to the visible appearance on the steak surface of numerous discrete holes or openings of varying size.

Texture defect refers to an undesirable increase in toughness and/or dryness, fibrousness, and watery nature of salmon examined in the cooked state.

83.5 FROZEN FISH FILLET BLOCKS

83.5.1 Product Description

Frozen fish blocks should be rectangularly-shaped masses made from a single species of fish flesh, and made from fillets or fillet pieces that are either skin-on and scaled or skinless. Blocks processed from skin-on fish flesh should be labeled as such. The blocks should not contain minced or comminuted fish flesh. The blocks should not be made by restructuring (reworking) pieces of fish blocks into the shape of a fish block.

83.5.2 Definitions of Defects

83.5.2.1 Dehydration. This defect refers to loss of moisture from the surface of a fish block during frozen storage. Affected areas have a whitish appearance.

Moderate dehydration masks the surface color of the product and affects more than 5% up to and including 15% of the surface area. If more then 15% of the surface area is affected, each additional 15% of surface area affected is another instance. Moderate dehydration can be readily removed by scraping with a blunt instrument.

Excessive dehydration masks the normal flesh color and penetrates the product. It affects more than 5% up to and including 10% of the surface area. If more than 10% of the surface area is affected, each additional 10% of surface area affected is another instance. Excessive dehydration requires a knife or other sharp instrument to remove.

83.5.2.2 Uniformity of Block Size. This defect refers to the degree of conformity to the declared size. It includes deviations from the standard length, width or thickness. Only one deviation for each dimension should be counted.

Moderate. A deviation of length and width of $\frac{1}{8}$ inch (0.32 cm) or more up to and including $\frac{1}{4}$ inch (0.64 cm). A deviation of thickness of $\frac{1}{16}$ inch (0.16 cm) or more up to and including $\frac{1}{8}$ inch (0.32 cm).

Excessive. If over $\frac{1}{4}$ inch (0.64 cm), each additional $\frac{1}{8}$ inch (0.32 cm) of length and width is another instance. If over $\frac{1}{8}$ inch (0.32 cm), each additional $\frac{1}{16}$ inch (0.16 cm) of thickness is another instance.

83.5.2.3 Underweight. Underweight refers to underweight deviations from the stated weight.

Slight. From 0.1 ounce (2.84 g) up to and including 1 ounce (28.35 g).

Moderate. Over 1 ounce (28.35 g) up to and including 4 ounces (113.4 g).

Excessive. If over 4 ounces (113.4 g), each additional 1 ounce (28.35 g) is another instance.

83.5.2.4 Angles. An acceptable edge angle is an angle formed by two adjoining surfaces whose apex (deviation from 90 degrees) is within 0.95 cm off a carpenter's

square placed along its surfaces. An acceptable corner angle is an angle formed by three adjoining surfaces whose apex is within 0.95 cm of a carpenter's square.

83.5.2.5 Improper Fill. This defect refers to voids, air pockets, ice pockets, ragged edges, bumps, depressions, damage, and embedded packaging material, each of which being greater than $\frac{1}{8}$ inch (0.32 cm) in depth, and that would result in product loss after cutting. It is estimated by determining the minimum number of 1 ounce (28.35 g) model units that could be affected adversely. For the purpose of estimating product loss, the 1 ounce (28.35 g) model unit should have the dimensions $4 \times 1 \times \frac{5}{8}$ inch ($10.16 \times 2.54 \times 1.59$ cm). The total number of model units that would be affected adversely is the number of instances.

83.5.2.6 Belly Flaps (Napes). May be either loose or attached to a fillet or part of a fillet. The maximum amount of belly flaps should not exceed 15% by declared weight of the block if this amount does exceed 15%, each additional 5% by declared weight is another instance.

83.5.2.7 Blood Spots. Each lump or mass of clotted blood greater than $\frac{3}{16}$ inch (0.48 cm) up to and including $\frac{3}{8}$ inch (0.95 cm) in any dimension is an instance. If a blood spot is larger than $\frac{3}{8}$ inch (0.95 cm), each additional $\frac{3}{16}$ (0.48 cm) is another instance.

83.5.2.8 Bruises. Includes distinct, unnatural, dark, reddish, grayish, or brownish off-colors due to diffused blood. Each instance is each bruise larger than $\frac{1}{2}$ square inch (3.32 cm^2) and less than $1\frac{1}{2}$ square inch (9.68 cm^2). For each bruise $1\frac{1}{2}$ square inch (9.68 cm^2) or larger, each additional complete 1 square inch (6.45 cm^2) is another instance.

83.5.2.9 Discoloration. Refers to deviations from reasonably uniform color characteristics of the species used, such as melanin deposits, yellowing, rusting or other kinds of discoloration of the fish flesh.

Moderate. A noticeable but moderate degree that is greater than $\frac{1}{2}$ square inch (3.23 cm^2) up to and including $1\frac{1}{2}$ square inch (9.68 cm^2) is one instance. If the discoloration is greater than $1\frac{1}{2}$ square inch (9.68 cm^2), each additional complete 1 square inch (6.45 cm^2) is another instance.

Excessive. An excessive degree of discoloration that is greater than $\frac{1}{2}$ square inch (3.23 cm^2) up to and including $1\frac{1}{2}$ square inch (9.68 cm^2) is one instance. If the discoloration is greater than $1\frac{1}{2}$ square inch (9.68 cm^2), each additional complete 1 square inch (6.45 cm^2) is another instance.

83.5.2.10 Viscera, Roe, and Lace. Viscera and roe refer to any portion of the internal organs. Each occurrence of viscera and roe is an instance. Lace (frill) is a piece of tissue adhering to the edge of a flatfish (*Pleuronectifonnes*) fillet. For each lace, each $\frac{1}{2}$ inch (1.27 cm) is an instance.

83.5.2.11 Skin. In skinless fish blocks, each piece of skin larger than $\frac{1}{2}$ square inch (3.23 cm^2) up to and including 1 square inch (6.45 cm^2) is an instance. For each piece of skin that is larger than 1 square inch (6.45 cm^2), each additional complete $\frac{1}{2}$ square

inch (3.23 cm^2) in area is another instance. For pieces of skin smaller than $\frac{1}{2}$ square inch (3.23 cm^2), the number of $\frac{1}{2}$ square inch (3.23 cm^2) squares fully or partially occupied after collecting these pieces on a grid is the number of instances.

83.5.2.12 Membrane (Black Belly Lining). Each piece of membrane (black belly lining) larger than $\frac{1}{2}$ square inch (3.23 cm^2) up to and including $1\frac{1}{2}$ square inch (9.68 cm^2) is an instance. For pieces of membrane (black belly lining) that are larger than $1\frac{1}{2}$ square inch (9.68 cm^2), each additional complete $\frac{1}{2}$ square inch (3.23 cm^2) in area is another instance.

83.5.2.13 Scales. For skin-on fillets that have been scaled, an instance is an area of scales over $\frac{1}{2}$ square inch (3.23 cm^2) up to and including $1\frac{1}{2}$ square inch (9.68 cm^2). If the area is greater than $1\frac{1}{2}$ square inch (9.68 cm^2), each additional complete 1 square inch (6.45 cm^2) is another instance. Loose scales are counted and instances are deducted in the same manner as for skinless fillets.

For skinless fillets, the first 5–10 loose scales is an instance. If there are more than 10 loose scales, each additional complete count of five loose scales is another instance.

83.5.2.14 Foreign Material. Any harmless material not derived from fish, such as packaging material. Each occurrence is an instance.

83.5.2.15 Bones. Includes pin bone and fin bone.

1. Each bone defect to a bone or part of a bone whose maximum profile is $\frac{3}{16}$ inch (0.48 cm) or more in length, or at least $\frac{1}{32}$ inch (0.08 cm) in shaft diameter or width, or, for bone chips, a longest dimension of at least $\frac{3}{16}$ inch (0.48 cm).
2. An excessive degree of bone defect is each bone whose maximum profile cannot be fitted into a rectangle, drawn on a flat, solid surface, that has a length of $1\frac{3}{16}$ inch (3.02 cm) and a width of $\frac{3}{8}$ inch (0.95 cm).

83.5.2.16 Fins or Part Fins. This defect refers to two or more bones connected by membrane, including internal or external bones, or both, in a cluster.

Moderate. Connected by membrane in a cluster, no internal bone.

Excessive. Connected by membrane in a cluster with internal bone.

83.5.2.17 Parasites

Metazoan parasites. Each such parasite or fragment of such a parasite that is detected is an instance.

Parasitic copepods. Each such parasite or a fragment of such a parasite that is detected is an instance.

83.5.2.18 Texture. The cooked product should have the textural characteristics of the indicated species of fish. Not included are any abnormal textural characteristics such as mushy, soft, gelatinous, tough, dry or rubbery.

Moderate. Moderately abnormal textural characteristics.

Excessive. Excessively abnormal textural characteristics.

83.6 FROZEN MINCED FISH BLOCKS

83.6.1 Product Description

Frozen minced fish blocks should be uniformly-shaped masses of cohering minced fish flesh, and may contain flesh from a single species or a mixture of species with or without food additives. The minced flesh should consist entirely of mechanically separated fish flesh processed and maintained in accordance with good commercial practice. This minced flesh should be made entirely from species that are known to be safe and suitable for human consumption.

83.6.2 Product Forms

83.6.2.1 Types

Unmodified: No food additives used.

1. Single species.
2. Mixed species.

Modified: Contains food additives

1. Single species.
2. Mixed species.

83.6.2.2 Color Classifications

1. White.
2. Light.
3. Dark.

83.6.2.3 Texture

Coarse. Flesh has a fibrous consistency.

Fine. Flesh has a partially fibrous consistency because it is a mixture of small fibers and paste.

Paste/Puree. Flesh has no fibrous consistency.

83.6.3 Definitions of Defects

83.6.3.1 Deteriorative Color. Discoloration from the normal characteristics of the material used. Deterioration can be due to yellowing of fatty material, browning of blood pigments, or other changes.

Slight deteriorative discoloration. A color defect that is slightly noticeable but does not seriously affect the appearance, desirability, or eating quality of the product.

Moderate deteriorative discoloration. A color defect that is conspicuously noticeable but does not seriously affect the appearance, desirability, or eating quality of the product.

Excessive deteriorative discoloration. A defect that is conspicuously noticeable and that seriously affects the appearance, desirability, or eating quality of the product.

83.6.3.2 Dehydration. A loss of moisture from the surfaces of the product during frozen storage.

Slight dehydration. Surface color masking, affecting more than 5% of the area, which can be readily removed by scraping with a blunt instrument.

Moderate dehydration. Deep color masking penetrating the flesh, affecting less than 5% of the area, and requiring a knife or other sharp instrument to remove.

Excessive dehydration. Deep color masking penetrating the flesh, affecting more than 5% of the area, and requiring a knife or other sharp instrument to remove.

83.6.3.3 Uniformity of Size. The degree of conformity to the declared contracted dimensions of the blocks. A deviation is considered to be any deviation from the contracted length, width, or thickness; or from the average dimensions of the blocks, physically determined, if no dimensions are contracted. Only one deviation from each dimension may be assessed. Two readings for length, three readings for width, and four readings for thickness will be measured.

Slight. Two or more deviations from declared or average length, width, and thickness up to $\pm\frac{1}{8}$ inch.

Moderate. Two or more deviations from declared or average length, width, and thickness from $\pm\frac{1}{8}$ inch to $\pm xx$ inch (variable, depending on products).

Excessive. Two or more deviations from declared or average length, width, and thickness over $\pm\frac{3}{8}$ inch.

83.6.3.4 Uniformity of Weight. The degree of conformity to the declared weight. Only underweight deviations are assessed.

Slight. Any minus deviation of not more than two ounces.

Excessive. Any minus deviation over two ounces.

83.6.3.5 Angles. An acceptable edge angle is an angle formed by two adjoining surfaces of the fish block whose apex is within $\frac{3}{8}$ inch (0.95 cm) of a carpenter's square placed along the surfaces of the block. For each edge angle, three readings will be made and at least two readings must be acceptable for the whole edge angle to be acceptable. An acceptable corner angle is an angle formed by three adjoining surfaces whose apex is within $\frac{3}{8}$ inch (0.95 cm) of the apex of a carpenter's square placed on the edge surfaces. Any edge or corner angle that fails to meet these measurements is unacceptable.

Slight. Two unacceptable angles.

Moderate. Three unacceptable angles.

Excessive. Four or more unacceptable angles.

83.6.3.6 Improper Fill. Surface and internal air or ice voids, ragged edges, or damage. Improper fill is measured as the minimum number of 1-ounce units that would be adversely affected when the block is cut. For this purpose, the dimensions of a 1-ounce unit are $4 \times 1 \times \frac{5}{8}$ inch.

Slight. One to three units adversely affected.

Excessive. More than three units adversely affected.

83.6.3.7 Blemishes. Pieces of skin, scales, blood spots, nape (belly) membranes (regardless of color), or other harmless extraneous material. One instance means that the area occupied by a blemish or blemishes is equal to a $\frac{1}{4}$ inch square. Instances are prorated on a per pound basis.

Slight. Five to 15 instances per pound.

Moderate. More than 15 but less than 30 instances per pound.

Excessive. 30 or more instances per pound.

83.6.3.8 Bones. Any objectionable bone or piece of bone that is $\frac{1}{4}$ inch or longer and is sharp and rigid. Perceptible bones should also be checked by their grittiness during the normal evaluation of the texture of the cooked product. Bones are prorated on a five pound sample unit basis.

Slight. One to two bones per five pound sample unit.

Moderate. Three to four bones per five pound sample unit.

Excessive. More than four bones; but not exceeding 10 bones; per five pound sample unit.

83.6.3.9 Flavor and Odor. Evaluated organoleptically by smelling and tasting the product after it has been cooked.

Good flavor and odor (essential requirements for a Grade A product) means that the cooked product has the flavor and odor characteristics of the indicated species of fish and is free from staleness, bitterness, rancidity, and off-flavors and off-odors of any kind.

Reasonably good flavor and odor (minimum requirements of Grade B product) means that the cooked product is moderately absent of flavor and odor characteristics of the indicated species of fish and is free from staleness, bitterness, rancidity, and off-flavors and off-odors of any kind.

Minimal acceptable flavor and odor (minimum requirements of a Grade C product) means that the cooked product has moderate storage induced flavor and odor, but is free from any objectionable off-flavors and off-odors that may be indicative of spoilage or decomposition.

83.6.3.10 Texture Defects. Judged on a sample of the cooked fish.

Slight. Flesh is fairly firm, only slightly spongy or rubbery. It is not mushy. There is no grittiness due to bone fragments.

Moderate. Flesh is mildly spongy or rubbery. Slight grittiness may be present due to bone fragments.

Excessive. Flesh is definitely spongy, rubbery, very dry, or very mushy. Moderate grittiness may be present due to bone fragments.

83.6.4 Additives

Minced fish blocks may be modified with food additives as necessary to stabilize product quality in accordance with federal requirements.

83.6.5 Hygiene

Fish material should be processed and maintained in accordance with federal requirements.

83.7 FROZEN RAW FISH PORTIONS

83.7.1 Product Description

The product described in this part should be clean, wholesome, shaped masses of cohering pieces (not ground) of fish flesh. The fish portions should be cut from frozen fish blocks, packaged in accordance with good manufacturing practice, and maintained at temperatures necessary for the preservation of the product. All fish portions in an individual package should be prepared from the flesh of one species of fish.

83.7.2 Styles

Style I: Skinless portions. Portions prepared from fish blocks that have been made with skinless fillets.

Style II: Skin-on portions. Portions prepared from fish blocks that have been made from demonstrably acceptable skin-on fillets.

83.7.3 Types

Type I: Uniform shaped. All portions in the sample are uniformly shaped.

Type II: Specialty cut. All portions not covered in Type I.

83.7.4 Definitions of Defects

83.7.4.1 Dehydration. The presence of dehydrated (water-removed) tissue in the portions. Slight dehydration is surface dehydration that is not color masking. Deep dehydration is color masking and cannot be removed by scraping with a blunt instrument.

83.7.4.2 Uniformity of Size. The degree of uniformity in length and width of the frozen portions. Deviations are measured from the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest in the sample.

83.7.4.3 Uniformity of Weight. The degree of uniformity of the weights of portions. Uniformity is measured by the combined weight of the two heaviest portions divided by the combined weight of the two lightest portions in the sample. No deductions are made for weight ratios less than 1.2 for Type I.

83.7.4.4 Blemishes. Skin (except for Style II), blood spots or bruises, objectionable dark fatty flesh, or extraneous material. Instances of blemishes refer to each occurrence measured by placing a plastic grid composed of $\frac{1}{4}$-inch squares (i.e., squares with an

area of $\frac{1}{16}$ square inch) over the defect area. Each square is counted as one whether it is full or fractional.

83.7.4.5 Bones. The presence of potentially harmful bones in a portion. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.

83.7.4.6 Texture Defects. Fish flesh and texture of skin in Style II refers to the absence of the normal textural properties of the cooked fish flesh and to the absence of tenderness of the cooked skin in Style II.

Normal textural properties of cooked fish flesh are tenderness, firmness, and moistness without excess water. Texture defects of the cooked flesh are dryness, mushiness, toughness, and rubberiness. Texture defects of the cooked skin in Style II are mushiness, rubberiness, toughness, and stringiness.

83.7.5 General Definitions

Small (overall assessment) refers to a condition that is noticeable but is only slightly objectionable.

Large (overall assessment) refers to a condition that is not only noticeable but is seriously objectionable.

Minor (individual assessment) refers to a defect that slightly affects the appearance and/or utility of the product.

Major (individual assessment) refers to a defect that seriously affects the appearance and/or utility of the product.

Net weight of the portions, if glazed, should be determined by the following method:

1. Weigh the portions with the glaze intact, which gives the gross weight.
2. Thaw the glaze from the surfaces of the product with flowing tap water.
3. Gently wipe off the excess water from the surfaces with a single water saturated paper towel.
4. Weigh the deglazed portions, which gives the net weight.

83.8 FROZEN RAW BREADED FISH STICKS

83.8.1 Product Description

Frozen raw breaded sticks should be clean, wholesome, rectangular-shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading. The sticks should be cut from frozen fish blocks, coated with a suitably wholesome batter and breading, packaged, frozen in accordance with good commercial practice, and maintained at temperatures necessary for the preservation of the product. Frozen raw breaded fish sticks should weigh up to and including $1\frac{1}{2}$ ounces, be at least $\frac{3}{8}$ inch thick, and their largest dimension be at least three times the next largest dimension. All sticks in an individual package should be prepared from the flesh of one species of fish.

83.8.2 Composition of the Product

Frozen raw breaded fish sticks should contain 72% by weight of fish flesh determined by the official end-product method. Fish flesh content may be determined by the on-line method provided that the results are consistent with the fish flesh content requirement of 72% by weight when verified by the official end-product method. Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any of the factors that may affect the relative fish flesh content.

83.8.3 Definitions

Selection of the sample unit. The sample unit should consist of 10 frozen raw breaded fish sticks taken at random from one or more packages as required. The fish sticks should be spread out on a flat pan or sheet and examined.

83.8.3.1 Examination of Sample, Frozen State

Condition of package. The presence in the package of loose breading and/or loose frost.

Ease of separation. The difficulty of separating sticks from each other or from the packaging material that have frozen together during the freezing.

Broken stick. A stick with a break or cut equal to or greater than one-half of the width of the stick.

Damaged stick. A stick that has been mashed, physically or mechanically injured, misshaped, or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of $\frac{1}{4}$ inch squares (i.e., squares with an area of $\frac{1}{16}$ square inch each) to measure the area of the stick affected. No deductions are made for damage of less than $\frac{1}{16}$ square inch.

Uniformity of size. The degree of uniformity in length and width of the frozen sticks. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest. Deductions are not made for overall deviations in length or width up to $\frac{1}{4}$ inch.

Uniformity of weight. The degree of uniformity of the weights of the sticks. Uniformity is measured by the combined weight of the two heaviest sticks divided by the combined weight of the two lightest sticks in the sample. No deductions are made for weight ratios of less than 1.15.

Cooked state. Is the state of the product after being cooked in accordance with the instructions accompanying the product. However, if specific instructions are lacking, the product for inspection is cooked as follows: the product is transferred while still in a frozen state into a wire-mesh frying basket that is large enough to hold the fish sticks in a single layer. The fish sticks are then cooked by immersing them for 2–3 min in a liquid or hydrogenated cooking oil at a temperature of 375°F (190°C). After cooking, allow the fish sticks to drain for 15 s and place the fish sticks on a paper napkin or towel to absorb excess oil.

83.8.3.2 Examination of Sample, Cooked State

Distortion. Is the degree of bending of the long axis of the stick. Distortion is measured as the greatest deviation from the long axis. Deductions are not made for deviations of less than $\frac{1}{4}$ inch.

Coating defects. Includes breaks, lumps, ridges, depressions, blisters or swells, and curds in the coating of the cooked product.

Breaks in the coating are objectionable bare spots through which the fish flesh is plainly visible.

Lumps are objectionable outcroppings of breading on the stick surface.

Ridges are projections of excess breading at the edges of the fish flesh.

Depressions are objectionable visible voids or shadow areas that are lightly covered by breading.

Blisters are measured by the swelling or exposed area in the coating resulting from the bursting or breaking of the coating.

Curd refers to crater-like holes in the breading filled with coagulated albumin.

Instances of these defects are measured by a plastic grid composed of $\frac{1}{4}$-inch squares ($\frac{1}{16}$ square inch in area). Each square is counted as one whether it is full or fractional.

Blemishes. Skin, blood spots or bruises, objectionable dark fatty flesh, or extraneous material. Instances of blemishes refer to each occurrence measured by placing a plastic grid composed of $\frac{1}{4}$-inch squares ($\frac{1}{16}$ square inch in area) over the defect area. Each square is counted as one whether it is full or fractional.

Bones. The presence of potentially harmful bones in a stick. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.

Texture defects of the coating. Absence of the normal textural properties of the coating, which are crispness and tenderness. Coating texture defects are dryness, sogginess, mushiness, doughiness, toughness, pastiness as sensed by starchiness or other sticky properties felt by mouth tissues and/or mealiness.

Texture defects of the fish. Absence of the normal textural properties of the cooked fish flesh, which are tenderness, firmness, and moistness without excess water. Texture defects of the flesh are dryness, mushiness, toughness, and rubberiness.

83.9 FROZEN RAW BREADED FISH PORTIONS

83.9.1 Product Description

Frozen raw breaded portions should be clean, wholesome, uniformly-shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading. The portions should be cut from frozen fish blocks, coated with a suitably wholesome batter and breading, packaged, frozen in accordance with good commercial practice, and maintained at temperatures necessary for the preservation of the product. Frozen raw breaded fish portions should weigh more than $1\frac{1}{2}$ ounces and be at least $\frac{3}{8}$-inch thick. Frozen raw breaded fish portions should contain not less than 75%, by weight, of fish flesh. All portions in an individual package should be prepared from the flesh of one species of fish.

83.9.2 Styles

Style I: Skinless portions. Portions prepared from fish blocks that have been made with skinless fillets.

Style II: Skin-on-portions. Portions prepared from fish blocks that have been made with demonstrably acceptable skin-on fillets.

83.9.3 Composition of the Product

Frozen raw breaded fish portions should contain 75% by weight of fish flesh. Fish flesh content may be determined by the on-line method provided that the results are consistent with the fish flesh content requirement of 75% by weight when verified by the official end-product method. Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors that may affect the relative fish flesh content.

83.9.4 Examination of Sample, Frozen State

Condition of package. The presence in the package of loose breading and/or loose frost.

Ease of separation. The difficulty of separating the portions from each other or from the packaging material.

Broken portion. A portion with a break or cut equal to or greater than one-half the width or length of the portion.

Damaged portion. A portion that has been mashed, physically or mechanically injured, misshaped, or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of $\frac{1}{4}$-inch squares (i.e., squares with an area of $\frac{1}{16}$ square inch each) to measure the area of the portion affected. No deductions are made for damage of less than $\frac{1}{16}$ square inch.

Uniformity of size. The degree of uniformity in length and width of the frozen portions. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest portions in the sample. Deductions are not made for overall deviations in length or width up to $\frac{1}{4}$ inch.

Uniformity of weight. The degree of uniformity of the weights of the portions. Uniformity is measured by the combined weight of the two heaviest portions divided by the combined weight of the two lightest portions in the sample. No deductions are made for weight ratios of less than 1.2.

Cooked state. Is the state of the product after being cooked in accordance with the instructions accompanying the product.

83.9.5 Examination of Sample, Cooked State

Distortion. Is the degree of bending of the long axis of the portion. Distortion is measured as the greatest deviation from the long axis. Deductions are not made for deviations of less than $\frac{1}{4}$ inch.

Coating defects. Includes breaks, lumps, ridges, depressions, blisters or swells, and curds in the coating of the cooked product.

Breaks in the coating are objectionable bare spots through which the fish flesh is plainly visible.

Lumps are objectionable outcroppings of breading on the portion surface.

Ridges are projections of excess breading at the edges of the portions.

Depressions are objectionable visible voids or shadow areas that are lightly covered by breading.

Blisters are measured by the swelling or exposed area in the coating resulting from the bursting or breaking of the coating.

Curd refers to crater-like holes in the breading filled with coagulated white or creamy albumin.

Instances of these defects are measured by a plastic grid composed of $\frac{1}{4}$-inch squares ($\frac{1}{16}$ square inch in area). Each square is counted as one whether it is full or fractional.

Blemishes. Skin (except for Style II), blood spots or bruises, objectionable dark fatty flesh, or extraneous material. Instances of blemishes refer to each occurrence measured by placing a plastic grid composed of $\frac{1}{4}$-inch squares ($\frac{1}{16}$ square inch in area) over the defect area. Each square is counted as one whether it is full or fractional.

Bones. The presence of potentially harmful bones in a portion. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.

Texture defects of the coating. Absence of the normal textural properties of the coating, which are crispness and tenderness. Defects in coating texture are dryness, sogginess, mushiness, doughiness, toughness, pastiness as sensed by starchiness or other sticky properties felt by mouth tissues and/or mealiness.

Texture defects of the fish flesh and texture of skin in Style II. Absence of the normal textural properties of the cooked fish flesh and to the absence of tenderness of the cooked skin in Style II. Normal textural properties of cooked fish flesh are tenderness, firmness, and moistness without excess water. Texture defects of the cooked flesh are dryness, mushiness, toughness, and rubberiness. Texture defects of the cooked skin in Style II are mushiness, rubberiness, toughness, and stringiness.

Minimum fish flesh content–End-product determination. The minimum percent, by weight, of the average fish flesh content of three frozen raw breaded portions (sample unit for fish flesh determination).

83.10 FROZEN FRIED FISH STICKS

83.10.1 Product Description

Frozen fried fish sticks should be clean, wholesome, rectangular-shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading, and partially cooked. The sticks should be cut from frozen fish blocks, coated with a suitably wholesome batter and breading, fried, packaged, frozen in accordance with good manufacturing practices, and maintained at temperatures necessary for the preservation of the product.

Frozen fried fish sticks should weigh up to and including $1\frac{1}{2}$ ounces, be at least $\frac{3}{8}$ inch thick, and their largest dimension be at least three times the next largest dimension. All sticks in an individual package should prepared from the flesh of one species of fish.

83.10.2 Composition of the Product

Frozen fried fish sticks should contain 60% by weight of fish flesh. Fish flesh content may be determined by the on-line method provided that the results are consistent with the fish flesh content requirement of 60 percent by weight when verified by the official end-product method. Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors that may affect the relative fish flesh content.

83.10.3 Definitions of Factors for Point Deductions

83.10.3.1 Examination of Sample, Frozen State

Condition of package. The presence in the package of free excess oil and/or loose breading and/or loose frost.

Ease of separation. The difficulty of separating sticks from each other or from the packaging material that have frozen together after the frying operation and during the freezing.

Broken stick. A stick with a break or cut equal to or greater than one-half of the width of the stick.

Damaged stick. A stick that has been mashed, physically or mechanically injured, misshaped, or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of $\frac{1}{4}$-inch squares (i.e., squares with an area of $\frac{1}{16}$ square inch each) to measure the area of the stick affected. No deductions are made for damage of less than $\frac{1}{16}$ square inch.

Uniformity of size. The degree of uniformity in length and width of the frozen sticks. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest. Deductions are not made for overall deviations in length or width up to $\frac{1}{4}$ inch.

Uniformity of weight. The degree of uniformity of the weights of the sticks. Uniformity is measured by the combined weight of the two heaviest sticks divided by the combined weight of the two lightest sticks. No deductions are made for weight ratios of less than 1.15.

Cooked state. Is the state of the product after cooking in accordance with the instructions accompanying the product.

83.10.3.2 Examination of Sample, Cooked State

Distortion. Is the degree of bending of the long axis of the stick. Distortion is measured as the greatest deviation from the long axis. Deductions are not made for deviations of less than $\frac{1}{4}$ inch.

Coating defects. Includes breaks, lumps, ridges, depressions, blisters or swells, and curds in the coating of the cooked product.

Breaks in the coating are objectionable bare spots through which the fish flesh is plainly visible.

Lumps are objectionable outcroppings of breading on the stick surface.

Ridges are projections of excess breading at the edges of the fish flesh.

Depressions are objectionable visible voids or shadow areas that are lightly covered by breading.

Blisters are measured by the swelling or exposed area in the coating resulting from the bursting or breaking of the coating.

Curd refers to crater-like holes in the breading filled with coagulated albumin.

Instances of these defects are measured by a plastic grid composed of $\frac{1}{4}$-inch squares ($\frac{1}{16}$ square inch in area). Each square is counted as one whether it is full or fractional.

Blemishes. Skin, blood spots, or bruises, objectionable dark fatty flesh, carbon specks or extraneous material. Instances of blemishes refer to each occurrence measured by placing a plastic grid composed of $\frac{1}{4}$-inch squares ($\frac{1}{16}$ square inch in area) over the defect area. Each square is counted as one whether it is full or fractional.

Bones. The presence of potentially harmful bones in a stick. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.

Texture defects of the coating. Absence of the normal textural properties of the coating, which are crispness and tenderness. Coating texture defects are dryness, sogginess, mushiness, doughiness, toughness, pastiness as sensed by starchiness or other sticky properties felt by mouth tissues, oiliness to the degree of impairment of texture, and/or mealiness.

Texture defects of the fish flesh. Absence of normal textural properties of the cooked fish flesh, which are tenderness, firmness, and moistness without excess water. Texture defects of the flesh are dryness, softness, toughness, and rubberiness.

83.11 FROZEN FRIED FISH PORTIONS

83.11.1 Product Description

Frozen fried fish portions should be clean, wholesome, uniformly-shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading and partially cooked. The portions should be cut from frozen fish blocks, coated with a suitably wholesome batter and breading, fried, packaged, frozen in accordance with good manufacturing practices, and maintained at temperatures necessary for the preservation of the product. Frozen fried fish portions should weigh more than $1\frac{1}{2}$ ounces and be at least $\frac{3}{8}$ inch thick. All portions in an individual package should be prepared from the flesh of one species of fish.

83.11.2 Composition of the Product

Frozen fried fish portions should contain 65% by weight of fish flesh. Fish flesh content may be determined by the on-line method provided that the results are consistent with

the fish flesh content requirement of 65% by weight when verified by the official end-product method. Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors that may affect the relative fish flesh content.

83.11.3 Examination of Sample, Frozen State

Condition of package. The presence in the package of free excess oil and/or loose breading and/or loose frost.

Ease of separation. The difficulty of separating portions from each other or from the packaging material that are frozen together after the frying operation and during the freezing.

Broken portion. A portion with a break or cut equal to or greater than one-half of the width or length of the portion.

Damaged portion. A portion that has been mashed, physically or mechanically injured, misshaped, or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of $\frac{1}{4}$-inch squares (i.e., squares with an area of $\frac{1}{16}$ square inch each) to measure the area of the portion affected. No deductions are made for damage of less than $\frac{1}{16}$ square inch.

Uniformity of size. The degree of uniformity in length and width of the frozen portions. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest. Deductions are not made for overall deviations in length or width up to $\frac{1}{4}$ inch.

Uniformity of weight. The degree of uniformity of the weights of the portions. Uniformity is measured by the combined weight of the two heaviest portions divided by the combined weight of the two lightest portions. No deductions are made for weight ratios of less than 1.20.

Cooked state. Is the state of the product after cooking in accordance with the instructions accompanying the product.

83.12 FRESH AND FROZEN SHRIMP

83.12.1 Product Description

The products should be clean wholesome shrimp that are fresh or frozen, raw or cooked.

83.12.2 Types

1. Chilled, fresh (not previously frozen).
2. Unfrozen, thawed (previously frozen).
3. Frozen individually (IQF), glazed or unglazed.
4. Frozen solid pack, glazed or unglazed.

83.12.3 Styles

1. Raw (uncoagulated protein).

2. Blanched (parboiled). Heated for a period of time so that the surface of the product reaches a temperature adequate to coagulate the protein.
3. Cooked. Heated for a period of time so that the thermal center of the product reaches a temperature adequate to coagulate the protein.

83.12.4 Market Forms

1. Heads on (head, shell, tail fins on).
2. Headless (only head removed: shell, tail fins on).
3. Peeled, undeveined, round, tail on (all shell removed except last shell segment and tail fins, with segments unslit).
4. Peeled, undeveined, round, tail off (all shell and tail fins removed, with segments unslit).
5. Peeled and deveined, round, tail on (all shell removed except last shell segment and tail fins, with segments shallowly slit to last segment).
6. Peeled and deveined, round, tail off (all shell and tail fins removed, with segments shallowly slit to last segment).
7. Peeled and deveined, fantail or butterfly, tail on (all shell removed except last shell segment and tail fins, with segments deeply slit to last segment).
8. Peeled and deveined, fantail or butterfly, tail off (all shell and tail fin removed, with segments deeply slit to last segment).
9. Peeled and deveined, western (all shell removed except last shell segment and tail fins, with segments split to fifth segment and vein removed to end of cut).
10. Other forms of shrimp as specified and so designated on the label.

83.12.5 Examination of Sample, Frozen State

Dehydration refers to a general drying of the shrimp flesh that is noticeable after any glaze and shell are removed. It includes any detectable change from the normal characteristics of the bright appearance of freshly caught, properly iced or properly processed shrimp.

Slight dehydration. Scarcely noticeable drying of the shrimp flesh that will not affect the sensory quality of the sample.

Moderate dehydration. Conspicuous drying of the shrimp flesh that will not seriously affect the sensory quality of the sample.

Excessive dehydration. Conspicuous drying that will seriously affect the sensory quality of the sample.

83.12.6 Examination, Sample, Fresh or Thawed State

Uniformity of size. The degree of uniformity of the shrimp in the container to determine their conformity to the declared count.

Black spots, improperly headed (throats), and improperly cleaned ends. The presence of any objectionable black or darkened area that affects the desirability or sensory quality of the shrimp, whether the market form is shell-on or peeled. Objectionable black spot refers to more than three instances of penetrating black spot that is visible but difficult to measure because of its small size (approximately the size of a pencil point); any areas larger than a pencil point that penetrates the flesh;

and aggregate areas of non-penetrating surface black spot on the shell or membrane that is equal to or greater than $\frac{1}{3}$ the area of the smallest segment. Assessments are made on individual shrimp. Throats are those portions of flesh and/or extraneous material from the head (cephalothorax) that remain attached to the first segment after heading.

Pieces of shrimp, broken or damaged shrimp

Shrimp pieces. Piece means, for a count of 70 or less unglazed shrimp per pound (0.45 kg), any shrimp that has fewer than five segments (with or without tail fins attached). Also, for a count of more than 70 unglazed shrimp per pound (0.45 kg), any shrimp that has fewer than four segments. Finally, any whole shrimp with a break in the flesh greater than $\frac{2}{3}$ of the thickness of the shrimp where the break occurs.

Broken shrimp. A shrimp having a break in the flesh greater than $\frac{1}{3}$ of the thickness of the shrimp.

Damaged shrimp. A shrimp that is crushed or mutilated so as to materially affect its appearance or usability.

Unusable material

Legs. Walking legs only, whether attached or not attached to the body (heads-on market form excepted).

Loose shell and antennae. Any pieces of shell or antennae that are completely detached from the shrimp.

Flipper. Any detached tail fin with or without the last shell segment attached, with or without flesh inside.

Extraneous material. Any harmless material in a sample unit that is not shrimp material.

Unacceptable shrimp and heads

Unacceptable shrimp. Abnormal or diseased shrimp.

Head. The cephalothorax, except for heads-on shrimp.

Inadvertently peeled and improperly peeled shrimp. The presence or absence of head, shell segment, swimmeret, or tail fin, which should or should not have been removed for certain market forms. (Shell-on shrimp with tail fins and/or telson missing is inadvertently peeled, but if the last segment of flesh is missing, the shrimp is damaged.)

Improperly deveined shrimp. The presence of the dark vein (alimentary canal) containing sand or sediment; or roe that should have been removed from peeled and deveined market forms. For shrimp of 70 count per pound (0.45 kg) or less, aggregate areas of dark vein or roe that are longer than one segment is a defect. For shrimp of 71 to 500 count per pound (0.45 kg), aggregate areas of dark vein or roe that are longer than two segments are a defect. (*Note*: This does not pertain to the last segment. For shrimp of over 500 count per pound (0.45 kg), dark vein or roe of any length is not a defect.)

83.12.7 Examination of Sample, Cooked State

Texture. The texture of cooked shrimp should be firm, slightly resilient but not tough, moist but not mushy. Texture as a defect refers to an undesirable toughness, dryness

or mushiness that deviates from the normal characteristics of the species when freshly caught, properly processed, and cooked.

Slight. Slightly tough, dry, but not mushy.

Moderate. Moderately tough, dry or mushy.

Excessive. Excessively tough, very dry or very mushy.

83.13 FROZEN RAW BREADED SHRIMP

The FDA has provided the following standards for frozen raw breaded shrimp.

83.13.1 Product Description

Frozen raw breaded shrimp should be whole, clean, wholesome, headless, peeled shrimp that have been deveined where applicable for the regular commercial species, coated with a suitably wholesome batter and/or breading. Whole shrimp should consist of five or more segments of unmutilated shrimp flesh. They should be prepared and frozen in accordance with good manufacturing practice and maintained at temperatures necessary for the preservation of the product.

Frozen raw breaded shrimp is the food prepared by coating one of the optional forms of shrimp with safe and suitable batter and breading ingredients. The food is frozen.

Not less than 50% of the breaded product is to be shrimp material as determined by the end product method.

The term shrimp means the tail portion of properly prepared commercial species of shrimp. Except for composite units, each shrimp unit is individually coated. The optional forms of shrimp are as follows.

Fantail or butterfly. Prepared by splitting the shrimp; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.

Butterfly, tail off. Prepared by splitting the shrimp; tail fins and all shell segments are removed.

Round. Round shrimp, not split; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.

Round, tail off. Round shrimp, not split; tail fins and all shell segments are removed.

Pieces. Each unit consists of a piece or a part of a shrimp; tail fins and all shell segments are removed.

The above information is categorized as follows.

83.13.2 Styles

Style I: Regular breaded shrimp. Frozen raw breaded shrimp containing a minimum of 50% of shrimp material.

Style II: Lightly breaded shrimp. Frozen raw breaded shrimp containing a minimum of 65% of shrimp material.

83.13.3 Types

Type I: Breaded fantail shrimp.

Subtype A: Split (butterfly) shrimp with the tail fin and the shell segment immediately adjacent to the tail fin.

Subtype B: Split (butterfly) shrimp with the tail fin but free of all shell segments.

Subtype C: Split (butterfly) shrimp without attached tail fin or shell segments.

Type II: Breaded round shrimp.

Subtype A: Round shrimp with the tail fin and the shell segment immediately adjacent to the tail fin.

Subtype B: Round shrimp with the tail fin but free of all shell segments.

Subtype C: Round shrimp without attached tail fin or shell segments.

Type III: Breaded split shrimp.

83.13.4 Definitions and Methods of Analysis

83.13.4.1 Fantail Shrimp. This type is prepared by splitting and peeling the shrimp except that for Subtype A, the tail fin remains attached and the shell segment immediately adjacent to the tail fin remains attached. Subtype B, the tail fin remains, but the shrimp are free of all shell segments. Subtype C, the shrimp are free of tail fins and all shell segments.

83.13.4.2 Round Shrimp. This type is the round shrimp, not split. The shrimp are peeled except that for Subtype A, the tail fin remains attached and the shell segment immediately adjacent to the tail fin remains attached. Subtype B, the tail fin remains, but the shrimp are free of all shell segments. Subtype C, the shrimp are free of all shell segments and tail fins.

83.13.4.3 Composite Units. Each unit consists of two or more whole shrimp or pieces of shrimp, or both, formed and pressed into composite units prior to coating; tail fins and all shell segments are removed; large composite units, prior to coating, may be cut into smaller units.

The batter and breading ingredients referred to are the fluid constituents and the solid constituents of the coating around the shrimp. These ingredients consist of suitable substances that are not food additives as defined by regulations. If they are food additives, as so defined, they are used in conformity with established regulations of the U.S. Food and Drug Administration. Batter and breading ingredients that perform a useful function are regarded as suitable, except that artificial flavorings, artificial sweeteners, artificial colors, and chemical preservatives, other than those specifically permitted, are not suitable ingredients for frozen raw breaded shrimp. Chemical preservatives that are suitable are:

1. Ascorbic acid, which may be used in a quantity sufficient to retard development of dark spots on the shrimp.
2. The antioxidant preservatives that may be used to retard the development of rancidity of the fat content of the food, in amounts within the limits prescribed by U.S. regulations.

The label should name the food, as prepared from each of the optional forms of shrimp specified, and following the numbered sequence of the following data.

1. "Breaded fantail shrimp." The word "butterfly" may be used in lieu of "fantail" in the name.
2. "Breaded butterfly shrimp, tail off."
3. "Breaded round shrimp."
4. "Breaded round shrimp, tail off."
5. "Breaded shrimp pieces."
6. Composite units.

If the composite units are in a shape similar to that of breaded fish sticks the name is "Breaded shrimp sticks"; if they are in the shape of meat cutlets, the name is "Breaded shrimp cutlets."

If prepared in a shape other than that of sticks or cutlets, the name is "Breaded shrimp _____," the blank to be filled in with the word or phrase that accurately describes the shape, but which is not misleading.

The word "prawns" may be added in parentheses immediately after the word "shrimp" in the name of the food if the shrimp are of a large size (e.g., "Fantail breaded shrimp (prawns)"). If the shrimp are from a single geographical area, the adjectival designation of that area may appear as part of the name (e.g., "Breaded Alaskan shrimp sticks").

The names of the optional ingredients used should be listed on the principal display panel or panels of the label with such prominence and conspicuousness as to render them likely to be read and understood by the ordinary individual under customary conditions of purchase. If a spice that also imparts color is used, it should be designated as "spice and coloring," unless the spice is designated by its specific name. If ascorbic acid is used to retard development of dark spots on the shrimp, it should be designated as "Ascorbic acid added as a preservative" or "Ascorbic acid added to retard discoloration of shrimp."

If any other antioxidant preservative is used, such preservative should be designated by its common name followed by the statement "Added as a preservative."

Frozen raw lightly breaded shrimp complies with the provisions of frozen raw breaded shrimp except that it contains not less than 65% of shrimp material and that in the name prescribed the word "lightly" immediately precedes the words "breaded shrimp."

83.13.5 Factors Evaluated on Unbreaded or Thawed Debreaded Product

Factors affecting qualities that are measured on the product in the unbreaded or thawed debreaded state are degree of deterioration, dehydration, sand veins, black spot, extra shell, extraneous material, and swimmerets.

1. *Dehydration*. The occurrence of whitish areas on the exposed ends of the shrimp (due to the drying of the affected area) and to a generally desiccated appearance of the meat after the breading is removed.

2. *Deterioration*. Any detectable change from the normal good quality of freshly caught shrimp. It is evaluated by noting in the thawed product deviations from the normal odor and appearance of freshly caught shrimp.
3. *Extraneous material*. Consists of nonedible material such as sticks, seaweed, shrimp thorax, or other objects that may be accidentally present in the package.
4. *Slight*. A condition that is scarcely noticeable but does affect the appearance, desirability, and/or eating quality of breaded shrimp.
5. *Moderate*. A condition that is conspicuously noticeable but that does not seriously affect the appearance, desirability, and/or eating quality of the breaded shrimp.
6. *Marked*. A condition that is conspicuously noticeable and that does seriously affect the appearance, desirability, and/or eating quality of the breaded shrimp.
7. *Excessive*. A condition that is very noticeable and is seriously objectionable.
8. *Halo*. An easily recognized fringe of excess batter and breading extending beyond the shrimp flesh and adhering around the perimeter or flat edges of a split (butterfly) breaded shrimp.
9. *Balling up*. The adherence of lumps of the breading material to the surface of the breaded coating, causing the coating to appear rough, uneven, and lumpy.
10. *Holidays*. Voids in the breaded coating as evidenced by bare or naked spots.
11. *Damaged frozen raw breaded shrimp*. Frozen raw breaded shrimp that have been separated into two or more parts or that have been crushed or otherwise mutilated to the extent that their appearance is materially affected.
12. *Black spot*. Any blackened area that is markedly apparent on the flesh of the shrimp.
13. *Sand vein*. Any black or dark sand vein that has not been removed, except for that portion under the shell segment adjacent to the tail fin when present.
14. *Extra shell*. Any shell segment(s) or portion thereof, contained in the breaded shrimp except the first segment adjacent to the tail fin for Type I, Subtype A, and Type II, Subtype A.

83.14 FROZEN RAW SCALLOPS

83.14.1 Product Description

Frozen raw scallops should be clean, wholesome, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species. The portion of the scallop used should be only the adductor muscle eye, which controls the shell movement. Scallops should be washed, drained, packed, and frozen in accordance with good manufacturing practice and maintained at temperatures necessary for the preservation of the product. Only scallops of a single species should be used within a lot.

83.14.2 Styles

Style I: Solid pack scallops. Scallops that are frozen together into a solid mass and are either glazed or not.

Style II: Individually quick frozen (IQF) pack. These scallops are individually quick frozen enabling individual scallops to be separated without thawing and are either glazed or not.

83.14.3 Types

Type 1: Adductor muscle.

Type 2: Adductor muscle with catch (gristle or sweet meat) portion removed.

83.14.4 Definitions

Dehydration. The loss of moisture from the scallops surface during frozen storage. A small degree of dehydration is color-masking but can be easily scraped off. A large degree of dehydration is deep, color-masking, and requires a knife or other instrument to scrape it off.

Extraneous materials. Pieces or fragments of undesirable material that are naturally present in or on the scallops, which should be removed during processing.

Minor. An instance of minor extraneous material includes, but is not limited to, each occurrence of intestines, seaweed, and so on, and each aggregate of sand and grit up to $\frac{1}{2}$-inch square and located on the scallop surface. Deduction points should be assessed for additional instances of intestines, seaweed, and so on, and aggregates of sand and grit up to $\frac{1}{2}$-inch square.

Major. An instance of major extraneous material includes, but is not limited to, each instance of shell or aggregate of embedded sand or other extraneous embedded material that affects the appearance or eating quality of the product.

Texture. The firmness, tenderness, and moistness of the cooked scallop meat, which is characteristic of the species.

Net weight. The total weight of the scallop meats within the package after removal of all packaging materials, ice glaze, or other protective materials.

83.15 FROZEN RAW BREADED SCALLOPS AND FROZEN FRIED SCALLOPS

83.15.1 Product Description

83.15.1.1 Frozen Raw Breaded Scallops. Frozen raw breaded scallops should be:

1. Prepared from wholesome, clean, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species, or scallop units cut from a block of frozen scallops that are coated with wholesome batter and breading.
2. Packaged and frozen according to good commercial practice and maintained at temperatures necessary for preservation.
3. Composed of a minimum of 50% by weight of scallop meat.

83.15.1.2 Frozen Fried Scallops. Frozen fried scallops should be:

1. Prepared from wholesome, clean, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species, or scallop units cut from a block of frozen scallops that are coated with wholesome batter and breading.

2. Precooked in oil or fat.
3. Packaged and frozen according to good commercial practice and maintained at temperatures necessary for preservation.
4. Composed of a minimum of 50% by weight of scallop meat.

83.15.2 Styles

The styles of frozen raw breaded scallops and frozen fried scallops include:

Style I: Random pack. Scallops in a package are reasonably uniform in weight and/or shape. The weight or shape of individual scallops is not specified.

Style II: Uniform pack. Scallops in a package consist of uniformly shaped pieces that are of a specified weight or range of weights.

83.15.3 Types

Type 1: Adductor muscle.

Type 2: Adductor muscle with catch (gristle or sweet meat) portion removed.

83.15.4 Definitions

Appearance. The condition of the package and ease of separation in the frozen state and continuity and color in the cooked state.

Condition of the package. Freedom from packaging defects and the presence in the package of oil, and/or loose breading, and/or frost. Deduction points are based on the degree of the improper condition as small or large.

Ease of separation. The difficulty of separating scallops that are frozen together after the frying operation and during freezing.

Continuity. The completeness of the coating of the product in the cooked state. A lack of continuity is exemplified by breaks, ridges, and/or lumps of breading. Each $\frac{1}{16}$ square inch area of any break, ridge, or lump of breading is considered an instance of lack of continuity. Individual breaks, ridges, or lumps of breading measuring less than $\frac{1}{16}$ square inch are not considered objectionable. Deduction points are based on the percentage of the scallops within the package that contain small and/or large instances of a lack of continuity.

83.15.5 Workmanship Defects

Workmanship defects refer to the degree of freedom from doubled and misshaped scallops and extraneous material. The defects of doubled and misshaped scallops are determined by examining the frozen product, while the defects of extraneous materials are determined by examining the product in the cooked state. Deduction points are based on the percentage by count of the scallops affected within the package.

Doubled scallops. Two or more scallops that are joined together during the breading and/or frying operations.

Misshaped scallops. Elongated, flattened, mashed, or damaged scallop meats.

Extraneous material. Pieces or fragments of undesirable material that are naturally present in or on the scallops, which should be removed during processing.

Minor. Includes intestines, seaweed, and each aggregate of sand and grit within an area of $\frac{1}{2}$-inch square.

Major. Includes shell, aggregate of embedded sand or other extraneous embedded material that affects the appearance or eating quality of the product.

83.15.6 Texture in the Cooked State

83.15.6.1 Texture of the Coating

Firm or crisp, but not tough, pasty, mushy, or oily.

Moderately tough, pasty, mushy, or oily.

Excessively tough, pasty, mushy, or oily.

83.15.6.2 Texture of the Scallop Meat

Firm, but tender and moist.

Moderately tough, dry, and/or fibrous or mushy.

Excessively tough, dry, and/or fibrous or mushy.

83.15.7 Character

Character refers to the texture of the scallop meat and of the coating and the presence of gristle in the cooked state.

Gristle. Gristle (type 2 only) is the tough elastic tissue usually attached to the scallop meat. Each instance of gristle is an occurrence.

Texture. The firmness, tenderness, and moistness of the cooked scallop meat and the crispness and tenderness of the coating of the cooked product. The texture of the scallop meat may be classified as a degree of mushiness, toughness, and fibrousness. The texture of the coating may be classified as a degree of pastiness, toughness, dryness, mushiness, or oiliness.

83.16 NORTH AMERICAN FRESHWATER CATFISH AND CATFISH PRODUCTS

83.16.1 Scope and Product Description

The descriptions apply to products derived from farm-raised, or from rivers and lakes, North American freshwater catfish of the following common commercial species and hybrids thereof:

1. Channel catfish (*Ictalurus punctatus*).
2. White catfish (*Ictalurus catus*).
3. Blue catfish (*Ictalurus furcatus*).
4. Flathead catfish (*Pylodictis olivaris*).

Fresh products should be packaged in accordance with good commercial practices and maintained at temperatures necessary for the preservation of the product. Frozen products should be frozen to 0°F (−18°C) at their center (thermal core) in accordance with good commercial practice and maintained at a temperature of 0°F (−18°C) or less.

The product may contain bones when the principle display panel clearly shows that the product contains bones.

83.16.2 Product Presentation

Catfish products may be presented and labeled as follows:

83.16.2.1 Types

1. Fresh.
2. Frozen.

83.16.2.2 Styles

1. Skin on.
2. Skinless.

83.16.2.3 Market Forms. These include, but are not limited to, the following:

1. Headed and gutted.
2. Headed and dressed are headed and gutted usually with the fins removed. This form may be presented with or without the dorsal spine and with or without the collar bone.
3. Whole fillets are practically boneless pieces of fish cut parallel to the entire length of the backbone with the belly flaps and with or without the black membrane.
4. Trimmed fillets are whole fillets without belly flaps.
5. Fillet strips are strips of fillets weighing not less than $\frac{3}{4}$ ounce.
6. Steaks are units of fish not less than $1\frac{1}{2}$ ounces in weight that are sawn or cut approximately perpendicular (30 degrees to 90 degrees) to the axial length or backbone. They should have two reasonably parallel surfaces. The number of tail sections that may be included in the package must not exceed the number of fish cut per package.
7. Nuggets are pieces of belly flaps with or without black membrane and weighing not less than $\frac{3}{4}$ ounce.

83.16.2.4 Bone Classifications

1. Practically boneless fillet.
2. Bone-in (fillet cut, with bones).

83.16.3 Definitions

Dehydration applies to all frozen market forms. It refers to the loss of moisture from the surface resulting in a whitish, dry, or porous condition.

Slight. Surface dehydration that is not color masking (readily removed by scraping) and affecting 3–10% of the surface area.

Moderate. Deep dehydration that is color masking, cannot be scraped off easily with a sharp instrument, and affects more than one percent but not more than 10% of the surface area.

Excessive. Deep dehydration that is color masking, and cannot be easily scraped off with a sharp instrument and affects more than 10% of the surface area.

Condition of the product applies to all market forms. It refers to freedom from packaging defects, cracks in the surface of a frozen product, and excess moisture (drip) or blood inside the package. Deduction points are based on the degree of these defects.

Slight. A condition that is scarcely noticeable but does not affect the appearance, desirability or eating quality of the product.

Moderate. A condition that is conspicuously noticeable but does not seriously affect the appearance, desirability, or eating quality of the product.

Excessive. A condition that is conspicuously noticeable and does seriously affect the appearance, desirability or eating quality of the product.

Discoloration applies to all market forms. It refers to colors that are not normal to the species. This may be due to mishandling or the presence of blood, bile, or other substances.

Slight. From $\frac{1}{16}$ square inch up to and including one square inch in aggregate area.

Moderate. Greater than one square inch up to and including two square inches in aggregate area.

Excessive. Over two square inches in aggregate area. Also, each additional complete one square inch is again assessed for points under this category.

Uniformity will be assigned in accordance with weight tolerances as follows (weight of portion: 0.75 to 4.16 ounces):

Moderate. Over $\frac{1}{8}$ ounce but not over $\frac{1}{4}$ ounce above or below declared weight of portion.

Excessive. In excess of $\frac{1}{4}$ ounce above or below declared weight of portion 4.17 to 11.20 ounces.

Moderate. Over $\frac{1}{8}$ ounce but not over $\frac{1}{2}$ ounce above or below declared weight of portion.

Excessive. In excess of $\frac{1}{2}$ ounce above or below declared weight of portion 11.21 to 17.30 ounces.

Moderate. Over $\frac{1}{8}$ ounce but not over $\frac{1}{8}$ ounce above or below declared weight of portion.

Excessive. In excess of $\frac{1}{8}$ ounce above or below declared weight of portion.

Skinning cuts apply to skinless market forms. It refers to improper cuts made during the skinning operation as evidenced by torn or ragged surfaces or edges, or gouges in the flesh that detract from a good appearance of the product.

Slight. From $\frac{1}{16}$ square inch up to and including one square inch in aggregate area.

Moderate. Over one square inch up to and including two square inches in aggregate area.

Excessive. Over two square inches in aggregate area. Also, each additional complete one square inch is again assessed for points under this category.

Heading applies to the presence of ragged cuts or pieces of gills, gill cover, pectoral fins or collar bone after heading. Deduction points will also be assigned when the product is presented with the collar bone and it has been completely or partially removed.

Slight. From $\frac{1}{16}$ square inch up to and including one square inch in aggregate area.

Moderate. Over one square inch up to and including two square inches in aggregate area.

Excessive. Over two square inches in aggregate area. Also, each additional complete one square inch is again assessed for points under this category.

Evisceration applies to all market forms. It refers to the proper removal of viscera, kidney, spawn, blood, reproductive organs, and abnormal fat (leaf). The evisceration cut should be smooth and clean. Deduction points are based on the degree of defect.

Slight. $\frac{1}{16}$ square inch up to and including one square inch in aggregate area.

Moderate. Over one square inch up to and including two square inches in aggregate area.

Excessive. Over two square inches in aggregate area. Also, each additional complete one square inch is again assessed for points under this category.

Fins refer to the presence of fins, pieces of fins or dorsal spines. It applies to all market forms except headed and gutted or headed and dressed catfish or catfish steaks. Deduction points will also be assigned when the product is intended to have the dorsal spine but it has been completely or partially removed.

Slight. Aggregate area up to and including one square inch.

Moderate. Over one square inch area up to and including two square inches.

Excessive. Over two square inches in aggregate area. Also, each additional complete one square inch is again assessed for points under this category.

Bones (including pin bone) apply to all fillet and nugget market forms. Each bone defect is a bone or part of a bone that is $\frac{3}{16}$ inch or more at its maximum length or $\frac{1}{32}$ inch or more at its maximum shaft width, or for bone chips, a length of at least $\frac{1}{16}$ inch. An excessive bone defect is any bone that cannot be fitted into a rectangle, which has a length of $1\frac{9}{16}$ inch and a width of $\frac{1}{8}$ inch. In market forms intended to contain bones, the presence of bones will not be considered a physical defect.

Skin refers to the presence of skin on skinless market forms. For semi-skinned forms, a skin defect is the presence of the darkly pigmented outside layers. Points will be assessed for each aggregate area greater than $\frac{1}{2}$ square inch up to and including one square inch.

Bloodspots refer to the presence of coagulated blood.

Bruises refer to softening and discoloration of the flesh. Both bloodspots and bruises apply to all market forms. Points will be assessed for each aggregate area of bloodspots or bruises greater than $\frac{1}{2}$ square inch up to and including one square inch.

Foreign material refers to extraneous material, including packaging material, not derived from the fish that is found on or in the sample. Each occurrence will be assessed.

Texture applies to all market forms and refers to the presence of normal texture properties of the cooked fish flesh, that is, tender, firm, and moist without excess water. Texture defects are described as dry, tough, mushy, rubbery, watery, and stringy.

Moderate. Noticeably dry, tough, mushy, rubbery, watery, stringy.

Excessive. Markedly dry, tough, mushy, rubbery, watery, stringy.

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84

Processing Frozen Seafoods

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84.1 Introduction 856
84.2 Nature of Fish Muscle 858
84.3 Preparation of Seafood Before Freezing 859
84.4 Freezing 860
84.4.1 Freezing Process 861
84.4.2 Freezing Time 861
84.4.3 Freezing Rate 862
84.4.4 Formation of Ice 862
84.4.5 Dimensional Changes 863
84.4.6 Desiccation 863
84.4.7 Freezing Methods and Equipment 864
84.4.7.1 Plate Freezers 864
84.4.7.2 Air Blast Freezers 865
84.4.7.3 Immersion Freezers 867
84.4.7.4 Evaporating Liquid Freezers 867
84.5 Packaging 868
84.5.1 General Requirements 868
84.5.2 Packaging Materials 869
84.5.2.1 Wrappers and Bags 869
84.5.2.2 Wooden Boxes 869
84.5.2.3 Rigid Aluminum Foil Packaging 869
84.5.2.4 Semirigid Plastic Packages 869

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

84.5.2.5 Tin and Composite Containers 869
84.5.2.6 Shipping Containers 869
84.6 Storage 870
84.6.1 Cold Store Design 870
84.6.2 Frost Heave 871
84.6.3 Insulation 871
84.6.4 Refrigeration System 871
84.6.5 Lighting 872
84.6.6 Layout 872
84.6.7 Jacketed Stores 872
84.6.8 Equipment 873
84.6.9 Operating Methods 873
84.7 Thawing 873
84.7.1 Surface-Heating Methods 874
84.7.2 Still-Air Thawing 874
84.7.3 Air Blast Thawing 874
84.7.4 Water Thawing 874
84.7.5 Vacuum Thawing 875
84.7.6 Double Contact Thawing 875
84.7.7 Internal Heating Methods 875
84.7.8 Electrical Resistance Thawing 875
84.7.9 High Frequency Thawing 876
84.8 Physicochemical Changes of Frozen Fish 876
84.9 Biochemical Changes in Frozen Seafoods 877
84.9.1 Postmortem Changes in Nucleosides 877
84.9.2 Postmortem Changes in Nucleosides and Lipid 877
84.9.3 Protein Denaturation of Frozen Fish 878
84.9.4 Effects of Freezing, Frozen Storage, and Thawing on Intrinsic Chemical Reactions 879
84.10 Microbiological Aspects during Freezing 880
84.11 Effects of Freezing Conditions on the Quality 881
84.11.1 Effects of Freezing, Frozen Storage, and Thawing 881
84.11.2 Change in Texture 883
84.11.3 Changes in Odor and Flavor 883
84.11.4 Effects of Storage Temperature 884
84.11.5 Effects of Fluctuation in Storage Temperature 885
84.11.6 Effect of Use of Additives 885
84.11.7 Effects of Packaging 886
84.12 Conclusion 887
References 888

84.1 INTRODUCTION

Fish and shellfish are perishable and easily spoil after harvesting due to postmortem chemical, physical, bacteriological, and histological changes. These degradation processes are usually accompanied by the gradual loss or development of different compounds that affect fish quality. The quality changes are highly affected by many factors, the most important of which is temperature. If fresh fish is not properly stored, exposure to ambient temperature can cause serious deterioration in fish quality. Commercially, icing or chilling continues to play a major role in slowing down bacterial and enzymatic

degradation of fish muscle. However, this process is not designed to eliminate totally the changes in quality, since it only offers protection for 2–3 weeks, depending on the species.

Freezing of food is an excellent method of preservation with wide applications. Freezing inhibits the growth of food spoilage and pathogenic organisms, and the low storage temperature greatly slows down the enzymatic and biochemical reactions that normally occur in unfrozen foods. It accomplishes these objectives in two ways: (1) by lowering the temperature of food, and (2) the removal of water by converting it into ice. Lowering the temperature to below freezing point inhibits the growth and activity of many but not all microorganisms and endogenous enzymes. Converting most of the water into ice with the concomitant increase in concentration of dissolved substances reduces the water activity of food to the point where no microorganisms can grow. Although biochemical reactions slow down at lower temperature, they still, unlike microbiological activities, progress even at low storage temperatures in some commercial freezer. In addition, conversion of water into ice initiates complex physical and physicochemical changes that can cause general deteriorative quality changes not ordinarily occurring in fresh foods. Pretreatments such as blanching, cooling, and so on, freezing, and storage conditions should therefore be carefully and individually selected for each product to minimize the effect of these deteriorative reactions.

In most commercial frozen foods, water, major component, in the tissue dissolves soluble cell components, while a small part is bound up in hydrates and in macromolecular colloidal complexes. In addition, most of the aqueous solution is part of the gel like or fiber like structures in the cell. The most obvious change that occurs on freezing is the solidification of water, which means that water is removed from its normal position within the tissues. It appears that removal of water from its normal position is only partly reversible upon thawing, leading to drip (exudate from thawed tissue) and other changes. It is very difficult to practically distinguish from superficial moisture or glaze and sometimes enhances susceptibility of microorganism invasion through the moist surfaces during thawing. Conversion of water into ice increases: the concentration of soluble cell components (in some cases to the point where they become saturated and precipitate), changes the pH of the aqueous solution, and consequently affects the amount of water that is involved in the colloidal complexes and in the gel-like and fiber-like structures. The concentrated cell components lead to a high concentration of electrolytes, some of which interpose themselves in the polypeptide chains of proteins, leading to protein denaturation. In living cells, this often leads to death (e.g., freezing and frozen storage causes a slight reduction in numbers of most microorganisms), but in foods, which usually consist of dead tissue prior to freezing, it can lead, during storage, to irreversible changes in texture (e.g., toughness in fish) and to undesirable biochemical reactions (enzymatically produced off-flavors). An understanding of freezing, therefore, involves physical, physicochemical, and biochemical aspects.

As mentioned above, freezing is an excellent process for keeping the original quality of foods, such as long term stored fish (commercially, up to 12 months or more). Freezing and subsequent frozen storage are particularly useful in making seasonal species of fish, like herring and mackerel, available all year round. In addition, freezing preservation is also applied in a number of different products made from various fish species. For example, tuna is frozen on board in the large commercial fishing vessels, brought to land, and then thawed for canning. In the production of various value-added fish products, freezing is applied to breaded and battered fish sticks, fillets, steaks, or nuggets. Likewise, high-quality fish are usually filleted, frozen, and eventually sold to consumers.

Ideally, there should be no distinguishable differences between fresh fish and frozen fish after thawing. If kept under appropriate conditions, fish in the frozen state can be

stored for several months or longer without appreciable changes in quality. However, it is now well recognized that deteriorative changes take place in fish and seafood during freezing, frozen storage, and thawing, which influence the quality of final products. Considerably more knowledge of the basic structure of fish muscle and its chemical composition are essential to the understanding of these changes occurred during processing.

84.2 NATURE OF FISH MUSCLE

Fish muscle has a unique arrangement of muscle fibers. It is divided into a number of segments called myotomes, which are separated from one another by a thin sheath of connective tissue called the myocomma or myoseptum. The number of myotomes in fish is dependent on the size of fish, while their diameters vary from head to tail (Love 1958). There are two major types of fish skeletal muscles, white and red. The red or dark muscle lies along the side of the body next to the skin, particularly along the lateral lines, and may comprise up to 30% of fish muscle, depending on the species (Green-Walker and Pull 1975).

Cells in red or dark muscle contain more lipids than that in white muscle (Buttkus and Tomlinson 1966; George 1962). They are basically employed for sustained swimming activities, functioning aerobically using lipids for fuel. In addition, red muscle has more mitochondria but less sarcoplasmic reticulum than white muscle (Patterson and Goldspink 1973). It has a large supply of oxygen and a high content of myoglobin, the colored compound that gives its red color. These characteristics, coupled with the presence of the large amount of lipid, particularly among the fatty species, present a serious problem of preservation because of increased susceptibility of this muscle to lipid oxidation. The red muscle of some species has also been reported to contain enzymes that are responsible for chemical reactions such as lipid oxidation and the conversion of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (FA) (Castells and others 1973a,b; Dyer and Hiltz 1974). The shape of red muscle area in different species varies considerably. Lean fish such as flounder, hake, sole, cod, pollock, and whiting have a very small amount of red muscle, which lies along the fish skin, whereas the fatty and semifatty fish species have larger areas of red muscle.

White muscle, on the other hand, constitutes the majority of fish muscle. Unlike red muscle, it has minimal myoglobin and a restricted blood supply. It is often referred to as the fast tissue (Shewfelt 1980).

Intermediate between these two types of muscle is intermixed red and white muscle, commonly referred to as "mosaic" muscle (Boddeke and others 1959). In some fish, this is a thin layer of muscle that separates the red from white muscle. However, in other fish, such as salmon, carp, and trout, this muscle is scattered throughout the body of the fish.

The chemical composition of fish varies depending on several factors, such as age, species, gender, maturity, method of catch, fishing grounds, and other seasonal and biological factors. Even within the same species, chemical composition may vary significantly. Generally, fish contain a considerable amount of protein, lipid, and water and small amounts of vitamins and minerals. Other components such as nonprotein nitrogenous compounds are also present in the muscle. These include urea, taurine, peptides, free amino acids, and nucleotides such as inosine and hypoxanthine (Konosu and others 1974).

These compounds, together with the macronutrients found in fish muscle, may be particularly important to fish processors, since they are frequently used as spoilage indices.

84.3 PREPARATION OF SEAFOOD BEFORE FREEZING

Most foods and food products are susceptible to attack by microorganisms and they are always contaminated by a variety of such organisms present in the food production chain. Foodstuffs are subjected to further contamination during preparation for freezing as a result of contact with the hands of factory staff during preparation, packaging and transport, and with air or water.

In view of the hazards to health and the effect of microbial contamination on quality, every effort must be made to reduce such contamination to a reasonable level during the preparation of foodstuffs for freezing. Throughout the world, frozen foods have very seldom been the cause of food poisoning incidents.

As pathogenic bacteria like *Salmonella* spp. and *Staphylococcus* spp. frequently derive from human sources, it is of vital importance that factory employees are aware of the basic concepts of good personal hygiene – the need for frequent washing and the wearing of clean clothes, overalls, hair covering, and so on. The use of rubber gloves and a protective mask for the mouth may be desirable in some instances. When gloves are worn they must be thoroughly cleaned and inspected before use. Medical supervision is advisable and in some cases should be made compulsory. Notices in lavatories should draw attention to personal hygiene, especially, the need for hand washing. Soaps, hand creams or dips containing antiseptic agents should be readily available.

Design of the building should ensure that both the buildings and drains are vermin proof. Interior walls, floor and ceilings should be finished with a nonflaking surface capable of withstanding detergents and sanitizers. All corners should be rounded to facilitate cleaning. The building should be large enough to house production equipment so that all sides of the equipment are accessible for cleaning. Entrance to the process area should be supplied with adequate washing facilities with foot-operated taps. Wood, almost impossible to sanitize, should not be used in contact with food. All windows should be both bird and fly proof.

Equipment usually becomes soiled with organic residues, which act as carriers of microorganisms. It should be designed and constructed to prevent hygienic hazard and permit easy and thorough cleaning. All surfaces should have a smooth, hard, waterproof finish. Cutting boards should be of a hard material; plastic is preferable to wood. Cleaning and disinfection of the food handling area, including equipment and utensils, should be carried out at frequent and regular intervals.

Waste materials should be frequently removed, in covered containers, from the working area during factory operation. Processes should be so separated – either in space or time – as to avoid recontamination of products in which the bacterial load has already been reduced. For cleaning purposes, an ample supply of potable water should be available. Chlorinated water is effective both for in-plant use (when concentrations of around 5–10 ppm are appropriate) and for sanitation of equipment and surfaces (when concentrations of around 100–200 ppm are used) but this should be followed by a rinse. Organic residues inactivate chlorine which, therefore, should only be used to sterilize already clean surfaces. Chlorine can be responsible for flavour loss or taint (due to the formation of chloramines or chlorophenols). Removal of the chlorine (by thiosulfate

addition, often combined with treatment in activated carbon towers) is practiced, especially in ice cream producing plants.

During the preparation of food for freezing, all efforts should be taken to avoid a build-up of an undesirable microbial population. For some foods, handling should take place at subambient temperature in temperature-regulated rooms, and where a heat treatment is a part of the processing this should be so severe that most of the microorganisms are killed. After heat treatment, the food should be promptly cooled to avoid multiplication of the surviving bacteria in the critical zone between 50° and 10°C. Cooling water, if used, should be chlorinated.

Proper organization of the various processes from the hygienic point of view is essential and a constant watch should be kept for lapses in hygiene. This should include a bacteriological control of the various stages in the processing line. Bacteriological methods are now available that give a good estimate of the bacterial load of the raw material and of food contact surfaces. Methods that give a rapid result are especially useful, as they supply plant management with information on the bacteriological state of products actually under preparation. The results of the examinations should be shown to the factory staff in order to make them comprehend the vital importance of hygiene in food production. Preferably, courses in food hygiene should be held at regular intervals for employees. The aim should be to give those engaged, in food production a thorough understanding of the hazard involved. The above considerations concerning personal hygiene, equipment, and preparation of foods also applies to handling of frozen foods in catering establishments. Thawing of frozen foods in these establishments should be completed as quickly as possible, and any storage of the food after thawing should be in a refrigerator.

84.4 FREEZING

Freezing is simply the crystallization of ice in muscle tissue and includes the consecutive processes of nucleation and growth. These processes are central to the effects of different freezing rates and subsequent effects on meat quality. Meat does not freeze immediately when its temperature drops below the freezing point and the latent heat (i.e., heat required during the phase change during crystal formation) has been removed. In other words there is a degree of supercooling. The greater the supercooling, the greater the number of nuclei formed. The number of nuclei is greatest in the extracellular space, and they are only formed within the cell when the rate of heat removal is higher. As soon as the nuclei form, they begin to grow by the accumulation of molecules at the solid/liquid interface. However, the way they grow depends on the microgeometry and the temperature distribution ahead of the freezing front, in a complex way, as a consequence of dendrite formation with supercooling in front of the dendrite growth. An important concept is characteristic freezing time, which is a measure of the local freezing rate and is defined as the time during which the point under consideration decreases from -1°C (freezing commences) to -7°C (when 80% of the water is frozen). The growth of extracellular ice crystals also takes place at the expense of intracellular water. This leads to partial dehydration of the muscle fibers and subsequent distortion. At high characteristic times (slow freezing), the ice crystals are larger and the tissue distortion is greater. The freezing process can rapidly minimize physical, biochemical, and microbiological changes in the food. This preservative effect is maintained by subsequent storage of the frozen food at a sufficiently cold temperature.

84.4.1 Freezing Process

The freezing process can be divided into three stages:

Stage 1: Cooling down from the initial temperature of the product to the temperature at which freezing begins. It must be borne in mind that the act of placing a product in a freezing apparatus does not render it "safe." Time will elapse before warm food passes out of the microbiologically hazardous temperature zone; this is particularly the case if freezing is carried out in slowly-moving air as in the freezing in bulk of products such as berries for subsequent jam manufacture.

Stage 2: This step covers the formation of ice in the products and extends from the initial freezing point to a temperature about $-5^{\circ}C$ colder at the center of the product. The major part of the freezable water will be converted to ice and this quite small reduction in temperature is accompanied by a massive enthalpy change.

Stage 3: Cooling down to the ultimate temperature for storage. When leaving the freezer, the frozen product will have a nonuniform temperature distribution; warmer in the center and coldest at the surfaces. Its average temperature will correspond to the value reached when the temperature of the product is allowed to equalize. In general, it is recommended to cool the product in the freezer to an equilibrium temperature of $-18^{\circ}C$ or colder. Product leaving the freezer with a warmer temperature will be stored for some time in relatively unfavorable conditions. Cooling down to storage temperature may take days or weeks.

84.4.2 Freezing Time

The effective freezing time is determined by (1) the initial and final temperature of the product and its change in enthalpy, and (2) the temperature of the heat transfer medium. The dimensions (especially the thickness) and shape of the product unit affect the overall heat transfer, which includes the surface heat transfer coefficient α and the heat conductivity λ characteristic of the product. When freezing by air blast α depends on the air velocity and the shape of the product. In an air blast freezer, the rate of freezing increases with increasing air velocity to an optimum value. The refrigeration load necessary to remove the heat produced by the fans increases with the cube of the air velocity; this factor should be taken into consideration when designing air blast freezers. It is important to direct the air circulation in such a way that the all product is equally exposed to the air current. In packaged food, the packaging material presents a resistance to the heat transfer, depending on its thickness and conductivity. This resistance is increased considerably if air is trapped between the package and the product.

Kato (1984) demosntrated the freezing time as a function of the thickness of fish fillets in packages frozen in a plate freezer ($\alpha = 200\ \mathrm{kcal/m^2 \cdot ^{\circ}C}$ including the packaging) and in a blast freezer with medium air velocity, $\alpha = 20\ \mathrm{kcal/m^2 \cdot ^{\circ}C}$) with heat removed from both sides of the package. A temperature of $-30^{\circ}C$ is assumed for the cooling medium (air, cold plates) and the process is considered to be complete when a temperature at the thermal center of $-15^{\circ}C$ is reached. The total freezing time is divided into two parts, one due to the influence of the internal conduction resistance ("λ-infl."), the other to the surface heat resistance, including package ("α-infl."). During plate freezing the heat conductivity of the product is the main factor determining freezing time. During air freezing the heat resistance of the surface (including packaging material) plays a dominant role for the product thickness encountered in practice.

Kato (1984) also showed that the freezing time in minutes for a 32 mm thick 450 g package of fish fillets in contact freezing is a function of the surface heat transfer coefficient. The total time is divided according to the influence of conduction resistance ("λ-infl.") and surface resistance, including package ("α-infl."). The type of packaging alone may increase the freezing time in a plate freezer by 2.5 times, and the surface heat transfer resistance by some four times. The freezing of packaged foods takes longer in an air blast freezer than in a plate freezer under comparable conditions. In the freezing of packaged food, where the λ-value is influenced by the air inside the package, the difference in freezing rate between plate and blast freezing diminishes as does the influence of packaging material.

84.4.3 Freezing Rate

Freezing must always be fast enough to minimize the development of microbiological and enzymatic changes in the product. A freezing process occupies a matter of days and will, in most cases, lead to deterioration in the frozen foodstuffs. In the past, the beneficial effect of very rapid freezing on the quality of frozen foods has been overestimated: within certain limits the rate of freezing does not materially affect the quality of most foods. This should not be interpreted to mean that the rate of freezing has no effect on the quality of frozen foods; most foods suffer from being frozen very slowly; a few foods demand ultra-rapid freezing. Fish and poultry seem to be more vulnerable to very slow freezing than most of other foods, and meat (beef, pork, and lamb) rather less. Strawberries and beans have a better texture and water holding ability if frozen ultra-rapidly while fruits and vegetables with a higher starch content such as peas are not as sensitive to freezing speed.

84.4.4 Formation of Ice

From a physical point of view, fish, land animal, and vegetable tissues can be roughly considered as dilute aqueous solutions. When they are chilled below 0°C, ice crystals form at a temperature characteristic of the product and the initial freezing point (FT), which is also the temperature at which the last ice crystals melt on thawing. The freezing point directly depends on the molar concentration of dissolved substances presented, but not on the water content. Fruits, for example, have high water content and a freezing point of −2 to −3°C, while fish contain less water, yet have a freezing point of about −1 to −2°C. The difference is due to the high sugar and acid content in fruits as compared with the low solute content of fish meat.

Supercooling is the phenomenon of reducing the temperature of a solution or material below its freezing point without crystallization occurring. Ice formation occurs during freezing only after a certain degree of supercooling has been achieved, and the formation of ice is accompanied by heating up of the supercooled product close to the freezing point. In commercial practice, the amount of supercooling is usually insignificant.

As the products are progressively cooled below their initial freezing point, more and more water will be turned into ice and the residual solutions will become more and more concentrated. If, at any time, the products are heated, some of the ice will be turned to water that will then dilute the residual solutions. The ratio of ice to residual solution in frozen foods is a function of temperature and initial concentration of solutes. At a temperature lower than −40°C, there is little or no measurable change in the amount of ice

presented in most frozen foods. The percentage ratio of freezing (RF) of frozen foods is usually estimated as follows:

$$\mathrm{RF}(\%) = 100 - [(\mathrm{FT}/\text{tem. frozen food}) \times 100]$$

where RF represents the percentage ratio of freezing and FT represents the freezing point of the frozen food.

84.4.5 Dimensional Changes

The volume change accompanying the conversion of pure water into ice is about 9%. The volume change of foods as a result of ice formation is less, about 6%, because only part of the water present is frozen and because some foods contain spaces. This volume change has to be taken into account in equipment design. In very fast freezing (e.g., immersion of large items in liquid nitrogen), it can lead to the build up of excessive pressure inside the product, causing breaking and shattering.

84.4.6 Desiccation

It is inevitable that a proportion of water of a product without packaging will evaporate during freezing. The faster the freezing, the smaller the amount of evaporated water. If the product is enclosed in a water-vapor proof package before freezing, no moisture will escapes from the packet. But, when there is an air gap (of the order of millimeters) between the surface of the product and the internal surface of the package frost may be deposited inside the package to the same extent as moisture evaporates from the product.

For products frozen unpackaged, moisture loss varies from 0.5 to 1.5% or more, depending on the temperature, rate and method of freezing, as well as the type of product. The colder the air temperature, the less moisture the air can absorb before it is saturated. Faster freezing methods lower the surface temperature of the product quickly to a value where the rate of moisture evaporation or sublimation is small. Where the surface of a product consists of a moisture-resistant layer (skin of the fish or fat on beef, for example), moisture losses are reduced in comparison with products with cut surfaces (fish fillets, hamburger patties, for example). Proper freezer design for a given product is, therefore, an important factor in minimizing moisture loss during freezing.

In commercial practice, mean freezing rates vary between 0.2 and 100 cm/h:

- 0.2 cm/h (slow freezing) for bulk freezing in blast rooms;
- 0.5 to 3 cm/h (quick freezing) for retail packages in air blast or plate freezers;
- 5 to 10 cm/h (rapid freezing) for individual quick freezing of small sized products, for example, in a fluidized bed;
- 10 to 100 cm/h (ultra rapid freezing) by spraying with or immersion in liquid gases.

For freezing of retail packages freezing rates faster than 0.5 cm/h and for individual quick freezing (IQF) products rates faster than 5 cm/h are considered satisfactory in most cases. Only very susceptible foods (such as tomatoes) may be improved by increasing the freezing to above 10 cm/h. Freezing rates should be taken with care to avoid cracking. When freezing larger units, such as beef quarters, with a mean freezing rate

of 0.1 cm/h a freezing time of 3–5 days is unavoidable and times up to 5 days are quite commonly used.

The rate at which freezing takes place can be considered both at micro and macro levels. At the micro level, freezing rate is described in terms of the speed with which the freezing front moves through the freezing object. At the macro level, the rate at which any given part of the object is cooled determines the temperature profile for that part, and thus has an important bearing on the biochemistry and microbiology of that part.

The undesirable changes in meat during freezing are associated with formation of large ice crystals in extracellular locations, mechanical damage by the ice crystals to cellular structures through distortion and volume changes, and chemical damage arising from changes in concentrations of solutes. The fastest freezing rates are associated with the least damage (Gruji and others 1993). Differences in freezing rate modify meat properties. Calvello (1981) discussed ice crystallization and its growth in meat tissues.

Freezing commences when the surface temperature of the meat reaches its freezing temperature. A continuous freezing front forms and proceeds from the exterior to the interior. Extracellular water freezes more readily than intracellular water because of its lower ionic and solute concentration. Slower freezing favors the formation of pure ice crystals and increases the concentration of solutes in unfrozen solutions. Intracellular solutions are often deficient in the nucleation sites necessary to form small ice crystals. Such conditions favor the gradual movement of water out of the muscle cells, resulting in a collection of large extracellular ice crystals and a concentration of intracellular solutes. Freezing damage arises from massive distortion and damage to cell membranes. Such effects have implications during thawing as the large extracellular ice crystals produce drip during thawing. The structural changes that occur also obliterate the recognizable muscle structure.

Fast freezing results in small ice crystal formation in both intracellular and extracellular compartments of the muscle and very little translocation of water. Drip loss during thawing is thus considerably reduced, and the surface reflects more light than that of slowly frozen meat. Consequently, the cut surface appearance is more acceptable.

84.4.7 Freezing Methods and Equipment

Freezing equipment may be divided into the following main groups with regard to the medium of heat transfer. These groups are metal: plate freezers; air (gaseous medium): blast freezers; liquid: immersion freezers; and evaporating liquid: liquid nitrogen and liquid fluorocarbon equipment. While blast freezers are used for all kinds of products packed or unpacked, blocks or IQF products, the plate freezer and immersion freezer accept only packaged product and evaporating liquid freezers are used only for IQF products.

84.4.7.1 Plate Freezers. In a plate freezer, the product is pressed by a hydraulic ram between metal plates. These plates have channels for the refrigerant. This arrangement gives very good heat transfer of metal contact. This high thermal efficiency is reflected in short freezing times, provided the product itself is a good heat conductor, as is the case with fish fillets or chopped spinach. It is important that the packets are well filled and the metal trays that are used to carry the packets are not distorted. The advantage of good heat transfer at the surface is gradually reduced with increasing thickness of the product. For this reason the thickness is often limited to a maximum of 50 mm. The

pressure from the plates maintained throughout the freezing process practically eliminates the "bulging" that may occur in air blast tunnels; the frozen packets will maintain their rectangular shape within close tolerances. There are two main types of plate freezers, horizontal plate freezers and vertical plate freezers:

- *Horizontal plate freezer.* Usually this type has 15–20 plates. The product is placed on metal trays, which are pushed in between the plates manually. This calls for a high labor content in the loading and unloading operation. In order to obtain automatic operation of a horizontal plate freezer, the whole battery of plates is movable up and down in an elevator system. At the level of a loading conveyor the plates are separated. Packages which have been accumulated on the conveyor are pushed in between these plates simultaneously discharging a row of frozen packages at the opposite end of the plates. This cycle is repeated until all frozen packages have been replaced. Then the space between the plates is closed and all plates are indexed up.
- *Vertical plate freezer.* The vertical plate freezer has been developed mainly for freezing fish at sea. It consists of a number of vertical freezing plates forming partitions in a container with an open top. The product is simply fed from the top. The frozen block is discharged either to the side, upwards or down through the bottom. Usually this operation is mechanized; the discharge of product often being assisted by a short hot gas defrost period at the end of the freezing cycle and the use of compressed air to force the product out.

84.4.7.2 Air Blast Freezers. Some foods, mainly bulk products such as beef quarters and fruits, for further processing are frozen in rooms with or without forced air circulation. Unless the room has been designed for freezing and equipped with suitable coolers and fans the freezing rate is very slow, resulting in an inferior quality for practically all products. If the room is also used for storage of frozen products the temperature of these products may rise considerably and the evaporators may frost up so quickly that the total refrigeration capacity is reduced below what is required to maintain the temperature of the store. Good commercial practices for air blast freezing uses include tunnel freezers, belt freezers and fluidized bed freezers.

- *Tunnel freezers.* In tunnel freezers the product is placed on trays, which stand in or pass through the tunnel in racks or trolleys one behind the other or singly. An air space is left between the trays. The racks or trolleys are moved in and out of the freezer by manual power or by a forklift truck (stationary tunnels), pushed through the tunnel with a pushing mechanism (push-through tunnel) or are carried through by driving equipment, chain drive, and so on (carrier freezer) or slid through (sliding tray freezer). Tunnels are also used for freezing hanging meat carcasses mostly carried on a suspension conveyor. Tunnel freezers are equipped with refrigeration coils and fans which circulate the air over the product in a controlled way. Guide devices, properly locating the trays of food, lead to uniform. Tunnel freezers are very flexible freezers. Products of every size and shape, Packaged or unpacked, can be frozen in stationary and push-through tunnels. Primarily they are used for freezing packaged products. Unpacked products tend to stick to the trays, which may cause weight losses and time-consuming handling in releasing, cleaning

and transport of the trays. To obtain free flow products, improved handling and an increase in the freezing rate, IQF is preferred.

- *Belt freezers*. Belt freezers are provided with a single belt. To increase throughputs and to reduce floor space, the belts may be positioned above each other which may run in the same or in opposite directions (multibelt-freezers), or as a spiral belt wound round a rotation drum stacking up to 30 tiers of the belt above each other (spiral-belt freezers). The belt, generally made of wire mesh, remains inside the freezer so that ancillary equipment for in and out feeding is necessary. Alternatively, the belt is carried to the outside as in some spiral-belt freezers. This arrangement has the advantage that the products can be placed on the belt in the processing room, where the operation can be supervised, before entering the freezer, and the product will remain undisturbed until removed at the outlet. System of baffles allows adjustment of the airflow. The belt is supported by rails and driven by passing round the rotating drum. Modern belt freezers have vertical airflow so that the air is forced through the product layer. In freezing, small products such as beans or cherries, good contact with all product particles is thus achieved. In single belt freezers with high air velocities, the products may be agitated. In all belt freezers, care should be taken to spread the product uniformly across the total belt width to avoid channeling, where the air stream bypasses the product. Belt freezers are used mainly for freezing unpackaged products, for example, IQF products. They are suitable for foods that need careful handling.
- *Fluidized bed freezer*. Fluidization occurs when particles of fairly uniform shape and size are subjected to an upward air stream. At an air velocity depending on the characteristics of the product, the particles will float in the air stream, each one separated from the other, but surrounded by air and free to move. In this state, the mass of particles behaves like a fluid. If the product is contained in an inclined trough fed at the higher end, the fluidized mass moves towards the lower end, as long as more products are added. The product is thus frozen and simultaneously conveyed by air without the aid of a mechanical conveyor.

The use of the fluidization principle has the following advantages when compared with a belt freezer:

- The product is always truly individually frozen (IQF). This applies even to products with a tendency to stick together, for example, shrimp, French style (sliced) green beans, sliced carrots, and sliced cucumber.
- Independence of fluctuations in load. If partly loaded the air distribution will be the same as for full load, that is, no hazard of channeling. If overloaded no product flows on to the floor.
- Reliability is improved when freezing wet products, because the deep fluidized bed can accept products with more surplus water.

An important factor in the overall operation economy of a blast freezer is the weight losses during freezing. Improperly designed equipment will have losses of 5% or more whilst a well-designed freezer normally operates with only 0.5–1.5% loss for unpackaged products. Parts of the weight losses are dehydration losses, which require particular

consideration. Low air temperatures and good heat transfer, that is, high air velocities, can minimize weight loss.

A freezing tunnel that is intended for packaged products should not, without due consideration, be used for thin unpackaged products, for example, fillets of fish. The result may be that the relation of coil surface to product surface is put out of balance so that air temperature in the tunnel rises resulting high weight losses. The coil may not be able to accommodate sufficient quantities of frost which results in reduced heat transfer or reduced airflow, both contributing to high weight losses.

It is important to note that in a vapor tight package containing a product that is not homogeneous, for example, beans or broccoli, the heat transfer inside the package is carried out by air. The heat transfer is very poor, because there is no air circulation. The result is evaporation of moisture, which actually may be greater than it would have been without the package. This moisture remains as frost on the inside surfaces of the package, so that it is not usually recorded as a weight loss. The influence on product quality is, of course, the same whether the dehydration is recorded or not.

Higher velocities of air give better heat transfer. However, it is not sufficient to just increase the fan power. The most important factor is to direct the air circulation in such a way that every product particle is efficiently and equally exposed to the air current. It is also important to study the conditions of the individual particles, because a close study may reveal surprising uneven airflow.

84.4.7.3 Immersion Freezers. For irregularly shaped products, for example, chicken, the best heat transfer is achieved in an immersion freezer. The immersion freezer consists of a tank with a cooled freezing medium, for example, a salt or propylene glycol solution. The product is immersed in this brine or sprayed while being conveyed through the tank. Immersion freezers are most commonly used for surface freezing of poultry to obtain a good color. The final freezing is affected in a separate blast tunnel or cold store. The latter alternative, however, involves quality hazards because of slow freezing of the core. However, the product must be protected by an absolutely tight, high quality packaging material. The brine on the package is washed off with water at the exit of the freezer.

84.4.7.4 Evaporating Liquid Freezers. Mainly two liquids or freezants are used, liquid nitrogen (LN2) and liquid fluorocarbon freezant (LFF).

- *LN2 freezer*. Liquid nitrogen at -196°C is sprayed onto a single belt freezer. The nitrogen evaporates and is allowed to escape to the atmosphere after the vapors have been used for precooling of the products. The very high freezing rate results in improved textures, particularly in certain fruits and vegetables while with other products there seems to be little quality advantage compared with other freezing methods. LN2-freezing may result in cracking of the product surface if sufficient precautions are not taken. Like immersion freezers LN2 freezers are often used only for surface freezing. If final freezing is to be carried out the LN2 consumption is of the order of 1.0–1.5 kg per kg of product which makes the operation rather expensive. In spite of this, the low investment and simple operation make this method economical for certain productions especially in-line processes.

- *LFF system*. The freezant is a specially purified dichlorodifluoromethane (fluorocarbon) which has a boiling point of $-30^{\circ}C$ at atmospheric pressure. The equipment consists of a container with openings at the top. The product is introduced into the container and dropped into a flowing stream of freezant. Owing to the extremely good heat transfer the surface is frozen instantaneously so the product may be stacked on the horizontal freezing belt, where it is sprayed with freezant until finally frozen.

A discharge conveyor brings the product up and out of the freezer. It is claimed that fluorocarbon leaves only small residues in most products. Experiments in this area are continuing. On contact with product the freezant evaporates. The vapors are recovered (with only a slight loss) by condensation on the surfaces of the refrigerator, the latter remaining in the container with only small losses to the atmosphere. There is no measurable product weight loss due to dehydration using this method.

84.5 PACKAGING

84.5.1 General Requirements

Not only must packaging used for frozen foods meet all the requirements of normal packaging but it must also meet requirements of packaging suitable for food such as:

- Chemical inertness and stability;
- Freedom from taint and odor;
- Freedom from toxic materials which may migrate into the food;
- Impermeability, or nearly so, to water vapor and other volatile constituents as well as to any odors from the surroundings;
- Suitability for use in automatic packaging systems;
- Suitable size and shape for display in retail cabinets;
- Protection from bacterial contamination and filth;
- Ease of opening;
- An attractive appearance.

In addition to these general requirements for food packages, frozen food packages should also:

- Be of such shape as to allow rapid freezing except for IQF;
- Permit volume expansion in the freezing process;
- Be impermeable to liquids and have good wet strength and resistance to water and weak acid;
- Be able to withstand low temperatures, not becoming excessively fragile at cold temperatures encountered during the freezing process;
- Not adhere to the contents in the frozen condition;
- Have a high reflectivity to reduce heat gain by radiation during display in retail cabinets;
- Be impervious to light as far as practicable;
- Surround the product closely, leaving the minimum of air entrapped, thus limiting sublimation during storage.

84.5.2 Packaging Materials

A wide variety of materials have been used in devising packaging systems for various frozen foods, for example, tinplate, paper, paper-board with a wax or plastic coating, aluminum foil, plastic film, thermoformed plastics and laminated combinations of these materials. Low permeability to water vapor is an important characteristic of packaging materials for frozen foods. Paper-board package for foods is generally in the form of folded cartons, either directly printed or provided with printed wrappers on the outside and in some cases with plastic coated liners. The coating or laminating materials including Wax blends (paraffin and microcrystalline compositions), plastics, for example, polyethylene or polypropylene (on one or both sides) and aluminum foil are commonly used.

84.5.2.1 Wrappers and Bags. Materials most commonly used are: waxed paper; hot melt or plastic coated paper; aluminum foil; coated cellulose films such as MSAT (moisture-proof, scalable, anchored and transparent) and plastic films, such as polyethylene (PE), polypropylene (PP), and polyvinylidene chloride (PVDC) films. Also of importance are the laminated materials built up from two or more of the abovementioned materials or other films. Common combinations are cellulose and PE films, sometimes with a PVDC coating. These materials are used as over wraps, liners and as single or double wall bags. The bags are either of the prefabricated type or are formed from roll stock on a filling machine. Particularly important are shrink-wrap materials because of their ability to adhere close to the product, leaving few if any air pockets. Shrink-wrap bags require evacuation of entrapped air before shrinking. Some of these bags can withstand boiling water and, therefore, the package can be used for end-cooking of the product before serving.

84.5.2.2 Wooden Boxes. Often used for fish, they require an inner liner or glaze on the product to guard against desiccation.

84.5.2.3 Rigid Aluminum Foil Packaging. These packages, in the form of trays, dishes and cups, are generally covered with a crimped-on aluminum sheet or a sheet of aluminum foil laminated to paper-board. Normally used for prepared foods and pastries, and so on, they allow rapid heating of the product in the package before serving.

84.5.2.4 Semirigid Plastic Packages. These are mostly manufactured from high density PE or PP in the form of trays and plates, covered by a lid; as with aluminum foil these can also be used for prepared foods requiring heating before serving, providing only gentle heat, such as steaming, is employed.

84.5.2.5 Tin and Composite Containers. These are used mainly for frozen juices that often have a mobile liquid phase even at cold store temperatures. A more recent development is to use coated paperboard in the body and aluminum for the ends, coupled with an easy opening device.

84.5.2.6 Shipping Containers. These are normally manufactured from different materials such as solid, corrugated fiberboard or vulcanized fiberboard paper, and plastics. They are often good heat insulators. Frozen-at-sea whole fish is frozen in blocks or individually. In both cases, the fish, if not packaged, should be glazed prior to storage to

minimize desiccation. Some fish is slabbed as skin-on fillets or made into blocks of skinned and boned fish for later cutting; both packs should be cartoned or bagged to minimize desiccation.

84.6 STORAGE

If the quality of frozen food is to be maintained during its storage life, the correct temperature must be selected for the expected period of storage. During the storage period, the following hazards to quality must be avoided:

- A low relative humidity in the cold store;
- Retention beyond the expected storage life;
- Fluctuations in temperature (both during storage and in the process of loading, unloading and dispatching vehicles);
- Physical damage to the product or packaging during the course of storage or handling;
- Contamination of the product by foreign bodies or vermin.

These hazards can be avoided by ensuring:

- That the design of the cold store is appropriate to the duty it will be required to perform, and is such that these hazards are as far as possible eliminated at the design stage;
- That operation methods, designed to avoid such dangers, are laid down and strictly adhered to.

Small fluctuations in temperature are normal and unavoidable. They should, however, be kept at a minimum both in amplitude and duration in order to minimize the amount of weight loss by drying and in-package desiccation.

84.6.1 Cold Store Design

The general design of a cold store is determined by the requirements for effective and safe handling of the merchandise, and a suitable storage climate for the products. The normal arrangement is that rooms are built side by side between road and railway loading banks, thus all rooms can communicate directly with the loading banks and traffic yards. Today, cold stores are frequently built with prefabricated concrete or steel structures. The insulation can be placed on the outside or the inside of the structure. Internal structure means that the insulation will form an unbroken envelope around the building. The insulation is well protected by the structure, internal installations are easy to fix, there is no hung insulated ceiling that can cause problems and extensions are very simple to carry out.

It is essential that considerable thought should go into the definition of the duties the cold store has to perform. A clear statement has to be prepared of the maximum and average daily activity expected to take place in the cold store:

- Quantity of each product to be received;
- Temperature at which each product will be received;

- Maximum number of operatives and trucks working in the cold store at any one time;
- Number of anticipated door openings;
- Maximum quantity to be out-loaded at any one time.

These considerations must be taken into account when calculating the maximum expected heat load. The temperature difference between the surface area of the cooling coils and the required room temperature should be as small as possible and not more than about 6°C.

84.6.2 Frost Heave

Frost heave under cold stores is prevented by a special under floor heating system or a ventilated space under the floor. The heating system may consist of an electrical mat or a pipe grid cast into the subfloor; glycol or oil is circulated in the pipes. The liquid is often heated by surplus heat from the refrigeration plant.

84.6.3 Insulation

The insulation represents a large percentage of the total cost for a normal cold store. It is, therefore, very important that it is designed from an economical point of view. However, one must also consider that the insulation value has an influence on the storage climate in that the transmission losses mean dry heat is entering the cold room. The choice of insulation system must be carried out carefully. The vapor barrier on the warm side must be completely water vapor tight, the insulation should contain no heat bridges and the internal cladding must be hard, hygienic and give a pleasant appearance. Today, most cold store insulations are carried out with prefabricated panels, slabs of polyurethane foam, expanded polystyrene, mineral fiber, or cork. Where special attention must be given to the risk of fire, the insulation is combined with a special firewall or the insulation is carried out with fiberglass between special insulation studs. The vapor barriers may consist of thin gauge aluminum, galvanized steel sheets, or heavy gauge polyethylene sheets. The joints are sealed with special sealing compounds. The internal cladding may be either profiled plastic, laminated galvanized steel sheet, or aluminum sheet. Good concrete kerbing and in some cases dunnage battens protect the internal finish and ensure that the merchandise is not stacked directly against the wall.

84.6.4 Refrigeration System

The refrigeration system must be designed with regard to the requirements of the climatic conditions for the stored merchandise. It must be adequate to allow for sufficient safety on peak days and summer conditions. The air coolers must be designed and located so that an even temperature can be maintained throughout the cold store even under severe conditions and without generating high air velocities in the cold store. Large evaporator surfaces and air distribution through air ducts or false ceilings will normally ensure this. Air ducts may be omitted if the cooling surface is divided on several cooler units distributed in the room so that the air velocity from the cooler fans is kept at a moderate level.

The most common refrigeration system for large cold stores is a two-stage compressor system with pump circulation of the cold refrigerant to the air coolers. The most common refrigerant is ammonia but halogenated hydrocarbons have also been used in some cases. For small cold stores a direct expansion one-stage compressor system using halogenated hydrocarbons is widely used. In order to improve safety and make control easier and cheaper most modern refrigeration plants are automated. The degree of automation may vary but normally the room temperature, compressor capacity, lubrication, cooling water, defrosting, pumps, fans, current and voltage of the main supplies, and so on, are controlled and supervised by a central control panel in the engine room.

84.6.5 Lighting

A cold store is a working place for fork lift drivers and others concerned with the handling of the products. Thus the lighting in the cold store must be good but at the same time it must be remembered that the lighting is adding to the heat load in a cold store. Lamps with a very high power/lighting ratio should be used. Mercury lamps are superior from this point of view and they are often used even if they can cause a slight discoloring of meat products during long storage. A normal cold store should have an average lighting of 100 lux at floor level and in break-up areas 200 lux.

84.6.6 Layout

The layout of the storage space should be such as to reduce to the maximum extent possible the ingress of warm air and the exposure of product to atmospheric temperatures. Where possible, product should be conveyed from factory areas into cold store by means of conveyors in insulated tunnels. There should be no facility for any accumulation of product in ambient temperature. If the operation is a palletized one, then palletization of the product should take place in the cold store in an area set aside for this purpose. Port doors should be provided so that the maximum amount of traffic in and out is handled in this fashion and the product is completely protected from temperature changes during loading/unloading operations. For safety reason, no glass should be allowed inside the cold store in any unprotected position. Translucent plastic visors should be placed around lamps or any other essential glass.

84.6.7 Jacketed Stores

The common jacketed stores has two two-speed fans. The classic operation for this type of jacketed stores is to have one fan circulating the air at lower speed and the other fan runs at the higher speed. This allows the air to enter the store through a system of movable vents that open via the effect of the increased air pressure. Thus, one can complete the cooling of foodstuffs that may have been subjected to warming up prior to being introduced into the cold store. Jacketed stores allow storage at near 100% relative humidity and at uniform and constant temperatures. These conditions, which greatly reduce weight losses of unpackaged foods and frost formation inside packaged frozen foods, are obtained by circulating the refrigerated air in a jacket around the load space to absorb the heat conducted through the insulation before it can enter the load space. This technique also increases the life and reduces the maintenance of the structure by preventing condensation and frost formation in the insulation.

84.6.8 Equipment

In equipping the store, care should be taken to choose equipment that is suitable to the product being handled and which minimizes the possibility of damage or contamination. Thus, timber pallets are suitable for properly packed products but lightly packed semiprocessed stock may need a pallet constructed in metal or some similar washable and less easily damaged material. It is likely, for economy reasons, that the store will be designed to maximize the use of height, and to this end some means of support for the product must be provided; for example, racks, pallet posts or pallet cages. The layout of pallets in the cold store should be such that damage to the products is minimized. Whilst accepting the need for maximum utilization of space, gangways and turnings should be wide enough for product to be moved without damage, whilst space should be allowed between lanes of pallets to permit the withdrawal or placing of a pallet when the adjacent lanes are full.

84.6.9 Operating Methods

It is important that everyone working in a cold store always bears in mind the prime objective of minimizing the exposure of products to ambient temperatures. Methods of handling and routes should be laid down which do not permit the product being placed in ambient temperature. If it is not possible to load a vehicle by means of a port door or some similar method that gives complete protection, the only alternative is to traverse a loading bank. Then the complete vehicle load should be assembled inside the cold store and conveyed direct to the vehicle without being placed on the loading bank. A similar procedure should apply in reverse for unloading. Doors should never be left open other than when personnel or goods are passing through them, and these should be as short a duration as practicable.

84.7 THAWING

Most frozen food processors find it necessary to thaw stock of frozen material in some of their operations, and if thawing is not carried out carefully, quality and yield can suffer. This section sets out the broad principles involved in the thawing techniques available, and indicates some of the problem areas. Irrespective of the procedure involved, heat energy must be supplied, most of it being required to melt the ice in the food. About 300 kJ are required to thaw 1 kg of white fish from a starting temperature of $-30^{\circ}C$. The figure for fatty herrings is smaller, about 250 kJ, because of the lower water content of herrings. Thawed materials spoil in the same way as their unfrozen equivalents and must be kept chilled until required; this can often be achieved conveniently by removing the product from the defroster just before thawing is complete so that the product has its own small reserve of cold.

There are two principal groups of thawing methods: those in which heat is conducted into the product from the surface; and those in which heat is generated within the product. In the first group, heat is applied to the surface of the product by exposing it to sources such as hot radiating surfaces, warm air, warm water, heated metal plates, or steam under vacuum. In the second group, heat is generated within the product by such means as electrical resistance, dielectric or microwave heating. Surface heating methods are much more commonly used than are internal heating methods.

84.7.1 Surface-Heating Methods

When using surface-heating methods, thawing time for a product decreases with:

- Decrease in physical size of the product;
- Increase in thermal conductivity (or, more precisely thermal diffusivity) of the thawed product;
- Increase in temperature difference between the surface of the product and its surroundings;
- Increase in the movement of the surrounding medium relative to the product surface;
- Increase in humidity of the surroundings.

Since the thermal diffusivity of thawed product is less than that of frozen, surface thawing methods suffer from the inherent disadvantage that resistance to heat transfer increases progressively once thawing has started. All surface-heating methods can take advantage of a programmed temperature difference between the surface and the surroundings. The temperature of the surroundings is arranged to start high, and to decrease as the surface warms up to a predetermined level, usually below the temperature where bacterial activity or surface damage could be a hazard to the quality of the food. Thawing times are greatly increased if the product is encased in packaging material. Thus packaging material should be removed where possible.

84.7.2 Still-Air Thawing

If thawing is conducted in still air, the air temperatures should not exceed 20°C and facilities for supplying heat to the room in which the product is laid out to thaw may be required. Air temperatures greatly in excess of the above should be avoided since the outer layer will warm up and spoil before the center is completely thawed. A typical block of sea frozen whole cod, 9 cm thick, will take up to 20 h to thaw under these conditions. This time can be reduced by immediately separating the fish without damage. Single fish, 10 cm thick, will take about 8–10 h to thaw.

84.7.3 Air Blast Thawing

A relative humidity of greater than 90% is advantageous both in reducing weight loss or shrinkage and also in obtaining a high level of heat transfer. Air velocities of 12–18 m/min at a temperature of 6–8°C for 3–5 days or 100 m/min for 2–2.5 days are needed for packages of boneless meat, whereas pork sides are normally thawed at 4–5°C for 2–4 days. Air speeds of the order of about 300 m/min at temperatures not exceeding 20°C with the air fully saturated with moisture will thaw sea frozen fish blocks 10 cm thick in 4 h. Higher air velocities at cool temperatures lead to surface desiccation while any higher temperature will lead to microbial growth on the heated surface.

84.7.4 Water Thawing

This method is not normally applicable to meat and with fish there is a risk that fillets or cut surfaces will become waterlogged and lose flavor but water thawing can be used

satisfactorily for frozen whole fish, even though there may be a slight loss of pigments. Usually there is a slight gain in weight, which is lost again when the fish is filleted. The temperature of the water being circulated around the frozen fish or sprayed on to the fish should be no warmer than 20°C and the water itself should flow at not less than 30 cm/min so as to obtain rapid thawing. The thawing time for a block of whole cod 10 cm thick in water at 20°C moving at about 120 cm/min is similar to that in an air blast defroster using humid air at the same temperature, that is, about 4 h. When frozen fish blocks are thawed in still water, water temperatures may, in the initial stages, marginally exceed 20°C, but the thawing should be arranged so that the fish surface temperature does not exceed 20°C.

84.7.5 Vacuum Thawing

In this method the product lies on racks inside a container from which the air has been evacuated. Water, usually at about 18°C is allowed to evaporate freely from heated vessels inside the container. In the absence of air, transfer of water vapor from a heated vessel to the product occurs readily, the water vapor giving up its latent heat on condensation. The advocates of this method claim faster thawing than other surface heating methods for products less than about 10 cm thick.

84.7.6 Double Contact Thawing

Plate frozen raw material lends itself particularly to plate thawing in an arrangement similar to that of a multiplate freezer, a liquid circulating through the plates (at a temperature not exceeding 20°C) providing the necessary heat. A 10 cm block of whole cod when thawed between plates at 20°C for 5 h is ready for filleting 3.5 h later, making a total of 8.5 h for complete thawing. Care must be taken not to allow distortion of the blocks to occur during cold storage since this will lead to poor contact with the plates during thawing. Thawing fluid or semifluid food in a vertical plate apparatus may be carried out using temperatures as high as 40–50°C as long as the melted material is allowed to run away from the plates continuously.

84.7.7 Internal Heating Methods

Internal heating methods rely on the use of applied electric fields which cause movement of the electric charges inherent in all products. The molecules of the product take up this energy of movement and the food warms up. The amount of heat generated in this way is strongly dependent on the electrical characteristics of the product. Since food is not usually homogeneous, there can be marked variations in the rate of heating of different parts of the food. Furthermore, for any particular component in the food, the rate of heating usually increases as the product thaws, making runaway heating a hazard. If these factors can be accommodated, the great advantage to be gained is extremely rapid, uniform thawing.

84.7.8 Electrical Resistance Thawing

In this method, the product is sandwiched between electrodes and an electric current is passed through the product. Some preliminary warming is usually necessary to achieve

good electrical contact and a satisfactorily high starting current. In practice this method has so far been used for thawing blocks of fish fillets up to 5 cm thick and weighing about 3 kg but it has not been found suitable for thicker blocks of fillets, blocks of whole fish (except herring) or single whole fish.

84.7.9 High Frequency Thawing

In the dielectric method, a high frequency electric field is applied to electrodes astraddling the product, but not physically in contact with it. The commonly used frequencies are either in the range 27–100 MHz (dielectric or high frequency heating) or 915–2450 MHz (microwave heating; where the energy is directed at the product enclosed in a chamber). The product must be reasonably homogeneous and regular in shape to achieve uniform heating. If the block is not homogeneous, or is irregular in shape, some parts may become overheated before the remainder is thawed. It has been found that if irregular blocks of fish are first immersed in water, thawing becomes uniform. The time taken for a 10 cm block of whole cod is typically just less than 1 h. Partial thawing by microwave is also used, thereby increasing the capacity of the thawing equipment. By going to the higher frequencies, the field strength can be substantially increased and thawing time reduced to a matter of minutes. Penetration into the food mass decreases so that the thickest block of meat which can be completely thawed at 2450 MHz is 3–4 cm.

84.8 PHYSICOCHEMICAL CHANGES OF FROZEN FISH

Freezing converts a large proportion of the water present in foods into ice and hence makes the remaining solution more concentrated in dissolved, colloidal and suspended substances. This increased concentration causes a change in acid-base equilibrium (pH) important in the stability of many colloids and suspensions. Shifts in pH (usually towards the acid side) of up to 1 pH unit have been observed under these conditions. A second result of this increased concentration is the precipitation of salts and other compounds that are only slightly soluble, such as phosphate. This can result in drastic pH changes (up to 2 pH units) and changes the salt composition of the aqueous solution in foods. These changes often affect the physicochemical systems in food irreversibly. It has been shown, for example, that lactic dehydrogenase, a muscle enzyme, and lipoproteins, an important egg yolk constituent, are irreversibly damaged by a pH decrease from 7 to 5 and by increased phosphate concentration during freezing.

Textural properties and the initiation and acceleration of several biochemical reactions depend on the physical chemistry of food constituents and hence are affected by the physicochemical changes brought about by freezing. Loss of water binding properties, resulting in drip, is an example of textural changes, while removal of enzymes from cell particles, allowing them free access to substrates in other parts of the cell, is an example of biochemical reactions initiated and accelerated by freezing. Other physicochemical changes in frozen foods are actomyosin changes in muscle, leading to toughening (fish) or dryness (poultry), loss of turgor in fresh fruits and vegetables, and gelation of egg yolk. Many physicochemical changes increase with increased salt concentration in the unfrozen phase, but will decrease with decreasing temperature as a result of the lower mobility of the salt in the unfrozen phase and the general effect of temperature on chemical

reactions. Consequently, physicochemical changes are most damaging in the range between the freezing point of a food and about $-10^{\circ}C$. It is important therefore to expose frozen foods for as short a time as possible to this temperature range, both during freezing and during thawing.

84.9 BIOCHEMICAL CHANGES IN FROZEN SEAFOODS

84.9.1 Postmortem Changes in Nucleosides

Fish muscle obtains energy by hydrolyzing adenosine triphosphate (ATP). At any one moment, its concentration is relatively small. During life it is quickly resynthesized using the energy produced when glycogen is oxidized to carbon dioxide and water. On death of the fish metabolism in the muscle continues for some time. Postmortem glycolysis, however, is a relatively inefficient process and it cannot maintain ATP at its level. Once ATP has fallen to a critical concentration, it can no longer prevent the major proportion of the muscle actin and myosin from crosslinking. This causes the loss of elasticity known as rigor mortis and usually a slow irreversible contraction. The continuing production of lactate and H^+ ions causes the pH of the muscle to fall from its *in vivo* value of about 7.2 to the so-called ultimate pH, which is usually about 5.5. A pH of 5.5 is near the isoelectric point of the muscle proteins, at which they have minimum water holding capacity and a consequent relatively high tendency to drip on thawing. A higher ultimate pH, therefore, means a greater water holding capacity than a lower pH. The quantity of glycogen present in the muscle at the moment of slaughtering will clearly determine how far the pH will fall during post mortem glycolysis. Like most chemical reactions, post mortem glycolysis is temperature dependent. It is generally found that the lower the temperature at which this process occurs, the slower is its rate. Thus if the carcass is maintained at body temperature after death, the rate of pH fall, of ATP depletion, and of rigor mortis onset is fast. If, however, the muscle is chilled quickly, these changes are slowed down and the water holding capacity of the muscle remains relatively high.

There is no practical cold shortening problem with fish properly chilled after catching. Fish muscle shows the least shrinkage if held at about $0^{\circ}C$. At warmer temperatures the shrinkage and weight loss are greater and they may be quite substantial for a fillet removed from the skeleton prerigor and kept at room temperature. One qualitative difference between fish and meat is the generally lower glycogen content in fish than in meat animals rested before death. Consequently, the post mortem fall of pH in fish is smaller and the resistance against surface bacterial growth less than in meat. In many fish species, therefore, bacterial spoilage is an overwhelming factor.

84.9.2 Postmortem Changes in Nucleosides and Lipid

The fat composition of fish differs markedly from that of meat, fish fat containing a higher proportion of polyunsaturated fatty acids. Although this factor may vary with species and is also influenced by dietary fat intake, it nevertheless implies that fish, in particular fatty fish, are very prone to development of rancidity by auto-oxidation. Such rancidity may even develop in fatty fish held before freezing, but it is particularly during storage of frozen fish that great care in packaging and the use of low temperatures are necessary to preserve quality.

84.9.3 Protein Denaturation of Frozen Fish

The proteins of fish muscle differ from those of meat especially in their higher susceptibility to cold store damage. Frozen storage of fish causes increase in drip loss upon thawing, toughness, coarseness and dryness on cooking, and loss of the desired glossy pellicle on smoking. These changes are highly associated with the so-called protein denaturation caused by freezing and subsequent storage. They are temperature dependent, the maximum rate of development being in the range -1 to $-5^{\circ}C$, which can be considerably slowed down by colder storage temperatures. The changes are mostly in the myofibrillar protein of fish muscle. In general, the sarcoplasmic proteins seem to be more stable on freezing and subsequent storage. This kind of protein denaturation is associated with the reaction of certain free fatty acids or their oxidized products on the myofibrillar proteins. Recently it has been found that the ultimate pH attained by fish can considerably affect texture. Thus, low pH in cod is associated with more pronounced toughness and larger drip loss on thawing.

Numerous studies indicated that denaturation of muscle proteins plays a dominant role in the quality changes (both physicochemical and sensory) of frozen stored fish muscle. The amount of extractable actomyosin decreased with the duration of storage, while no significant change in sarcoplasmic proteins was observed during frozen storage of cod and other fish (Dyer 1951; Dyer and Morton 1956; Dyer and others 1956).

Previous studies suggested that the aggregation of muscle proteins occurred during frozen storage. These study results include electron microscopic analyses (Ohnishi and others 1978), decreases in actomyosin peak (20s–30s), areas on ultracentrifugal analysis (Noguchi and Matsumoto 1970; Oguni and others 1975) and viscosity of soluble actomyosin with duration of storage (Oguni and others 1975; Ueda and others 1962a,b). In addition to aggregation, dissociation of F-actomyosin into f-actin and myosin also occurred. It appeared that the dissociated f-actin, as thin filaments, became entangled and aggregated and that the dissociated myosin monomers folded into globular form. At advanced stages of freeze denaturation, large masses with diffuse outlines were frequently found indicating the formation of aggregation complex of actin and myosin (Matsumoto 1980).

ATPase activity of actomyosin and myosin, another property of myosin related to its contractile function, also decreased with the increase of frozen storage (Chen and others 1989; Jiang and others 1988a,b, 1989; Noguchi and Matsumoto 1970; Oguni and others 1975; Ueda and others 1962a,b).

During the initial frozen storage, it appears that both myosin and actin undergo denaturation, while denaturation of tropomyosin and troponin was observed during prolonged frozen storage (Irisa and others 1978).

The involvement of SH group in the denaturation of muscle proteins during frozen storage has been emphasized by Buttkus (1970, 1971), Chen and others (1989), and Jiang and others (1988a,b, 1989). Therefore, the changes appear to be the result of rearrangements of disulfide bonds from intramolecular to intermolecular through a sulfhydryl-disulfide interchange reaction.

Myofibrils, a systematically organized complex of myofibrillar proteins, undergo some structural changes during frozen storage of fish. The most noticeable change is the fusion of the myofibrils as illustrated by cell fragility method (Love and Mackay 1962; Love and others 1965) and fragmentation into short pieces at the Z-bands (Jarenbäck and Liljemark 1975a,b; Jiang and others 1990, 1992; Tokiwa and Matsumiya 1969). More recently, studies have been done on the denaturation of enzymes during frozen storage

(Gould 1965; Tappel 1966). Inactivation of enzymes with globular molecule was considered to be due to the unfolding of intramolecular structure (Hanafusa 1973).

One of the most prevalent chemical reactions to occur in fish muscle during freezing and frozen storage is the complex phenomenon of protein denaturation. It has been postulated that the rupturing of different bonds in the native conformation of proteins in frozen fish is followed by side-by-side aggregation of myofibrillar proteins, specifically myosin, brought about by the formation of intermolecular cross-linkages (Connell 1960; Sikorski 1978). It is also believed that the significant decrease observed in the center-to-center distance between the thick filaments of the A-band of the sarcomere after prolonged frozen storage favors the formation of crosslinkages between molecules and stiffens the fibers (Jarenbäck and Liljemark 1975a,b). Such intermolecular crosslinkages result in aggregation (Matsumoto 1979), which leads to the formation of high molecular weight polymers (Childs 1973; Lewin 1974) and subsequent denaturation of myosin during frozen storage.

84.9.4 Effects of Freezing, Frozen Storage, and Thawing on Intrinsic Chemical Reactions

When frozen fish are subjected to excessively prolonged cold storage at temperatures above $-30^{\circ}C$, a series of intrinsic chemical reactions occurs in fish tissues. These reactions include protein denaturation, breakdown of TMAO and lipid oxidation.

Quite obviously, protein denaturation during frozen storage produces extensive textural changes and deterioration in fish. These changes are more pronounced in some species of fish, specifically the gadoids, and are related to another intrinsic chemical reaction, the breakdown of TMAO. After death, TMAO is readily degraded to DMA and FA through a series of reactions. This conversion of TMAO to DMA and FA is typically observed in frozen gadoid species such as cod, hake, haddock, whiting, red hake, and pollock (Castells and others 1973a,b).

The presence of air (oxygen) affects DMA and FA formation. It has been suggested that oxygen may actually inhibit the reaction by interacting with metal ions, which otherwise would accelerate the TMAO degradation (National Marine Fisheries Services 1986). Lundstrom and others (1981) observed that red hake (*Urophysis chuss*) minces stored in the absence of oxygen showed more rapid DMA and FA formation than red hake fillets stored in air. Likewise, the presence of air (oxygen) in packaged white hake (*Urophysis tenuis*) significantly prolonged the shelf-life of the frozen samples (Santos and Regenstein 1990).

TMAO degradation to DMA and FA was enhanced by the presence of an endogenous enzyme (TMAOase) in the fish tissues, as observed in cod muscles by Amano and Yamada (1965a,b). However, evidence also exists which demonstrates that breakdown of TMAO to DMA and FA is nonenzymic in nature (Spinelli and Koury 1979; Tarr 1958). The breakdown of TMAO, whether enzymatically or nonenzymatically induced, is believed to produce destabilization and aggregation of proteins. It has been suggested that TMAOs breakdown product, FA, may produce crosslinking of muscle proteins (Matsumoto 1979) due its high reactivity. Clearly, the presence of FA is not the only factor involved in textural changes during frozen storage. However, with certain species of fish, it appears to be of primary importance.

Another chemical reaction generally associated with quality changes during freezing, frozen storage, and thawing is lipid oxidation (oxidative fat rancidity), one of the major causes of frozen shelf-life reduction. The extent of oxidation in fish lipids varies with

the quantity and the type of lipids in the fish muscle, that is, fatty species are more prone to oxidation than lean species, and species with more highly unsaturated fatty acids are less stable than the other species. When oxidative rancidity progresses sufficiently, it leads to the development of obvious off-tastes and odors, resulting in reduced shelf life. Changes in fish lipids may be related to changes in protein during frozen storage. Several reports indicate that the unstable free radical intermediates formed during autoxidation attack the protein molecules, leading to the formation of protein free radicals (Castells and others 1973a,b; Karel and others 1975). Another possible mechanism for reaction between oxidized lipids and proteins occurs through stable oxidation products (Konosu and others 1974; Shenouda 1980) increasing the hydrophobicity of proteins, making them less water soluble. Free fatty acids (FFA) formed during autoxidation produce indirect effects on textural degradation by promoting protein denaturation (Colmonero and Borderias 1983). FFA are believed to bind myofibrillar proteins, specifically actomyosin, rendering it unextractable (Shenouda 1980; Sikorski and others 1975).

84.10 MICROBIOLOGICAL ASPECTS DURING FREEZING

Freezing and subsequent storage will kill some of the microorganisms present in the unfrozen material, but this is a slow and variable process depending, in part, upon the nature of the food. Thus freezing cannot be relied upon to substantially reduce bacterial contamination present in the foodstuff. The hygienic state of the product before freezing is consequently all-important. Storage at temperatures colder than $-12^{\circ}C$ inhibits microbial growth and therefore is one effective method of preserving food against microbial spoilage. Three aspects of bacteria in frozen seafoods including resistance to freezing and frozen storage, multiplication in frozen and thawed foods will be considered.

Some pathogens are more resistant to freezing than are ordinary spoilage organisms. Most of the common pathogenic bacteria are Gram-negative. This group is more sensitive to freezing, frozen storage and thawing than are the Gram-positive spoilage organisms. Even when microbial growth is completely inhibited, the frozen product can still deteriorate due to the activity of the released microbial enzymes that can still catalyze undesirable biochemical reactions in the food. When the handling of fish before freezing is improper, there is the danger that microorganisms may have released sufficient enzymes and toxin to affect the quality of the frozen product. For example, if lipases are produced before freezing, they can cause marked hydrolysis of fats in fatty fish even when stored at $-30^{\circ}C$. If fish has been held at relatively high temperatures before freezing, any pathogens present could multiply and some may produce toxins. The latter will survive freezing and constitute a health hazard. A product destined for freezing should receive the same degree of hygienic handling as that which is to be stored at chill temperature.

Several psychrotrophic microorganisms can multiply at freezing temperatures. In practice, bacterial growth does not occur below temperatures of $-10^{\circ}C$. This is probably due to the increasing concentration of soluble salts and organic compounds in the unfrozen water that will decrease the water activity of this fraction.

Upon thawing, frozen foods will spoil almost at the same rate as would be expected from unfrozen products with the same microbial population maintained at similar temperatures. Condensation of moisture on the surface of the product should be avoided as during thawing it may cause acceleration of microbial growth.

Pathogenic bacteria may grow and produce toxin in food without rendering the food unpalatable. They will be occasionally observed in any food even hygienically prepared. Contamination even with small numbers of pathogenic bacteria during preparation of foods for freezing should therefore be avoided as far as practicable. Competition between different types of bacteria is important if food that will be further prepared after thawing is stored in the thawed state before such preparation. A food lacking a normal flora of spoilage organisms, but contaminated with a few pathogens is more likely to present a health hazard than the same food contaminated to the same degree with normal spoilage flora. In the case of frozen foods allowed to thaw slowly, the psychrotrophic flora is likely to dominate and may so alter the substrate as subsequently to inhibit or slow down the multiplication of any pathogens present when thawing is complete. Packaging exerts little effect on the spoilage pattern, even vacuum packing causes a negligible increase in the growth rate of anaerobes like *Clostridium botulinum* during storage after thawing.

84.11 EFFECTS OF FREEZING CONDITIONS ON THE QUALITY

It is readily apparent that spoilage changes in fresh fish occur most commonly as a result of bacterial activity. The species of bacteria vary according to storage temperature. In fish stored in ice, *Alteromonas*, *Achromobacter*, and *Flavobacter* spp. predominate. At temperatures between 35 and 55°C, *Micrococcus* and *Bacillus* spp. constitute the main microflora. Some of these microorganisms produce very active proteolytic enzymes, which produce odor, flavor, and textural problems. When fish and seafood are frozen, the microorganisms present in their tissues are generally inactivated. Thus, during frozen storage, microbiological changes in fish tissue are usually minimal. Microorganisms not destroyed by the freezing process generally do not grow and in some cases die off slowly. Although some microorganisms survive storage at very low temperatures, their activities are suppressed, and bacterial numbers may be considerably reduced if recommended temperatures are maintained (Hultin 1985). The temperature below which microbial growth is considered minimal ranges from -10 to -12°C (Dellino 1986). Microorganisms, however, that survive and remain inactivated during frozen storage resume growth when the fish is thawed and may then lead to microbial spoilage of the thawed product. Frozen fish are far from sterile and cannot therefore be considered a microbiologically safe product. The microbial activities in fish after thawing depend on the degree of freshness of the raw material, the natural microflora in the fish tissues, and the thawing technique utilized.

The effects of various freezing conditions on quality and shelf stability of frozen fish and seafood have received considerable attention recently. Studies have dealt with either the stability of the frozen product as related to storage temperature and fluctuation in storage temperature or the effectiveness of food additives in providing shelf stability to frozen products.

84.11.1 Effects of Freezing, Frozen Storage, and Thawing

One problem encountered during handling, freezing, and storage of fish is the difficulty in retaining the color and appearance of the meat. Changes in color and appearance of fish occur even immediately after catch. Blood pigments become noticeably discolored to

various degrees after some period of time. The natural pigments in the fish meat contributes to its color. They also play an important role in the color changes and vary from one species to another. These pigments are subjected to considerable oxidation when the fish is frozen and stored. This then results in meat color darkening to either dark brown or, in some cases, black. This discoloration occurs especially when the fish is stored for an extended period of time.

Some fish, like tuna, develop discoloration during frozen storage, reportedly due to oxidation of myoglobin to met-myoglobin in fish blood (Bito 1964). Other species, such as salmon, swordfish, and shark, also exhibit color changes during storage. Salmon has a pink meat, but when subjected to oxidation its color slowly fades and, in extreme cases, may completely disappear after prolonged storage. Swordfish, on the other hand, develops green discoloration beneath its skin during frozen storage, which according to Tauchiya and Tatsukawa (1954) is due to the development of sulf-hemoglobin, a product of oxidation. Shark flesh also discolors and occasionally develops off-odors during storage, most probably as a result of the presence of high amounts of trimethylamine oxide. Interestingly, these marked differences in the color and appearance of frozen fish are quite noticeable in fish sold either as steaks or as fillets. This is especially true when cross-sectional cuts of the fish are made, which permits a comparison of the color of the exposed fish surface with that of the inner portion.

In shrimp, the rapid formation of black pigments, widely known as melanosis, occurs within a few hours after death and is enhanced by exposing the shrimp to air (oxygen). It can occur within just 2–12 h of exposure. The oxidation reaction leading to the formation of these black pigments can occur at 0°C, but at −18°C, no visible spots were detected at up to 3 months of storage (Reilly and others 1984). Below this temperature, it is believed that melanosis can still positively occur. It should be noted, however, that although black spots do not necessarily make shrimp unfit for human consumption, such discoloration is usually associated with spoilage, resulting in a decrease in market value. In other shellfish, such as crab and lobster, the development of blue or black discoloration, otherwise known as blueing, is one of the most troublesome problems. Blueing may occur after freezing or during frozen storage, or it may appear after thawing and subsequent air exposure or even shortly after cooking. Needless to say, these changes in color and appearance of fish and shellfish significantly affect consumer acceptance. When consumers select frozen fish, if these products can be seen through the packaging material used, the color and appearance of the frozen product provide an indication as to its degree of quality.

Thawing also influences the color and appearance of frozen fish and, inevitably, its consumer acceptability. Depending on the thawing technique used, discoloration may occur in fish and other seafood. For instance, when shrimp are thawed at temperatures higher than 0°C, black discoloration or melanosis may occur. This is due to the unnecessary exposure of the shrimp to air, leading to oxidation. A phenomenon known as "shimi" occurs in frozen-thawed fish meat. "Shimi" are the undesirable blood spots observed in the belly portion of carp upon thawing and are also the distinguishable spots tainting frozen-thawed tuna meat (Bito 1984). The latter condition is probably due to the blood vessels that remain in unbled tuna meat prior to freezing. When thawed, these blood vessels produce unsightly spots in the meat.

It is possible to determine if the product has been properly thawed and then refrozen. This is particularly noticeable in packaged frozen fish, where spaces on the sides of the package may be filled with a frozen cloudy liquid, known as thaw drip. Such muscle drip was originally attributed to the rupturing of cell walls caused by ice crystal formation

during freezing, resulting in excess drip during thawing. However, it has been postulated that drip or exudate formation is directly related to the capacity of the fish protein to hold moisture (Ciarlo and others 1985). This unsightly exudate from fish muscle indicates, among other things, inappropriate handling, prolonged ice storage prior to freezing, frozen storage at inappropriate cold-storage temperatures, or improper thawing. If not properly controlled, freezing, frozen storage, and thawing generally result in quality changes in fish and seafood that in most cases render the product unacceptable to consumers.

Changes in the texture, odor and flavor of fish and seafood affect their palatability. Fresh fish have a distinct succulence and a delicate odor and flavor, which are characteristic of the species. These attributes change noticeably when fish is frozen and stored for prolonged periods of time. Interestingly, the changes that influence the palatability of frozen fish and seafood can all be measured organoleptically, and to some extent chemically.

84.11.2 Change in Texture

Frozen fish gradually loses its juiciness and succulence after freezing and subsequent frozen storage. Such textural changes, reportedly caused by protein denaturation (Shenouda 1980; Sikorski and others 1975; Sikorski 1978, 1980), are more pronounced in some species of fish, specifically the gadoids. In these species, the chemical breakdown of TMAO to DMA and FA and the subsequent crosslinking of FA to muscle protein (Matsumoto 1979) produce the textural breakdown in the gadoids and result in a cottony or spongy texture. Fish muscle that has undergone such changes tends to hold its free water loosely like a sponge. When eaten, the fish muscle loses all its moisture during the first bite, and subsequent chewing results in a very dry and cottony texture.

In some species devoid of TMAO-degradation products, muscle fibers also tend to toughen and to become dry during freezing and storage. This is particularly true for most of the nongadoid species and for crab, shrimp, and lobster when stored for prolonged periods. In contrast, the effect of the thawing method on the texture of fish muscle basically depends on the product form. For instance, whole fish, when thawed, exhibits less textural change than filleted fish, basically as a result of the presence of the backbone, which serves as structural support for the flesh. In terms of the effect of the thawing method, it has been reported that microwave thawing results in higher gel strength of minced samples, when compared to samples thawed under running water (20°C) and samples thawed at room temperature (Jiang and others 1985). Consequently, the extent of textural changes depends upon the species of fish and upon the condition of handling, freezing, duration of frozen storage, and the thawing method used.

84.11.3 Changes in Odor and Flavor

Other important changes that affect the palatability of frozen fish include changes in the flavor and odor of fish and seafood. Fish are often described as having a "fishy" odor and flavor. Although the term sounds unpleasant, it can also be used to describe the pleasing taste and odor characteristics of freshly caught fish. Such pleasant, palatable characteristics may be retained as long as the fish are promptly and properly frozen, stored, and thawed. However, the transformation of these attributes to unpleasant and

unacceptable traits occurs very rapidly in some fish species, particularly the fatty fish species.

Changes in the delicate flavor of fish and seafood generally occur in three distinct phases during frozen storage:

- The gradual loss of flavor due to loss or decrease in concentration of some flavor compounds (Dyer 1951; Licciardello and others 1980);
- The detection of neutral, bland, or flat flavor;
- The development of off-flavors due to the presence of compounds such as the acids and carbonyl compounds that are products of lipid oxidation.

These phases, however, only apply to those species with originally delicate, sweet, and meaty flavors. Other species, such as hakes, have an originally bland flavor (Licciardello and others 1980), but develop off-flavors during prolonged frozen storage.

Changes in odor occur in two phases: the loss of characteristic odor and the development of off-odors, which render the frozen product unacceptable. Generally, fish and seafood initially have a fresh, seaweed odor, which can be retained even after freezing and frozen storage. However, gradually such odor is lost, and eventually an unpleasant odor is given off, particularly when abused with inappropriate storage temperature. The development of unpleasant odor is due either to lipid oxidation, a reaction more apparent among the fatty fish species that results in the production of a strong oily, blow oily, or rancid odor, or to the degradation of TMAO, which leads to the production of an unpleasant ammonia odor. Other species such as white hake (*Urophysus tenuis*) initially give off weak odors of sweet, boiled milk, but when frozen storage is extended, hake assumes weak off-odors (often described as milk jug odor) followed by a sour milk odor.

84.11.4 Effects of Storage Temperature

The apparent effects of storage temperature on shelf-life stability of frozen fish are related to protein denaturation and lipid oxidation. The effects of temperature on protein denaturation have been comprehensively studied (Dyer and Morton 1956; Hanson and Olley 1965). Maximum denaturation is reported to occur at $-4^{\circ}C$ in cod muscle (Dyer 1951), while changes in extractable proteins in haddock have been found to be greatest at -2 to $-6^{\circ}C$ (Reay 1983).

The rate of lipid oxidation and the accumulation of FFA were observed to increase with temperature (Connell 1975). In a study using various species of fish, it was observed that maximum production of FFA due to enzymic activities of lipases occurred at -12 to $-14^{\circ}C$ (Olley and others 1962), while the maximum rate of lipid hydrolysis was detected at temperatures just below freezing (Lovern and Olley 1962). Storage at much lower temperatures can, therefore, prolong the shelf life of frozen fish. For example, cod stored at $-160^{\circ}C$ showed no detectable deterioration after 6 months of storage (Connell 1975). Even at -65 and $-50^{\circ}C$, frozen samples exhibited very few changes after 9 months of storage. Such observations also suggest that low storage temperature limits the problems associated with protein denaturation and lipid oxidation during frozen storage.

Several studies have been conducted in an attempt to determine the shelf life of frozen fish at different temperatures and to establish storage temperatures that can minimize

quality deterioration in specific groups of fish. Poulter (1978) reported that *Rastrelliger brachysoma* (club mackerel) stored at −10°C remained acceptable until the ninth month of storage, whereas samples kept at −30°C were rejected after 12 months of storage. *Scomber scombrus* (Atlantic mackerel) stored unwrapped at −18°C were rejected after 3 months of storage, while samples at −26°C remained acceptable until the sixth month of storage (Ke and others 1976). Early rejection of fatty species at relatively low temperatures is reportedly due to the development of rancid flavor and odor. Several studies have also reported the same dependence of shelf-life for different fish species on temperature (King and Poulter 1985; Santos and Regenstein 1990; Screenivasan and others 1976). Clearly, fish composition has an appreciable effect on shelf-life stability of frozen fish. For instance, in a comprehensive study using different fish species, it was found that fatty fish such as mackerel, salmon, herring, sprat, and trout had a shelf life of 2–3 months at −18°C, whereas lean fish such as cod, flounder, haddock, ocean perch, and pollock exhibited storage stability of up to 4 months at the same storage temperature (Bramnaes 1969). Based on several studies, it is recommended that those species most susceptible to oxidative rancidity be stored at very low temperatures (at least −29°C) while species less susceptible to rancidity should be stored at temperatures between −18 and −23°C (Wheaton and Lawson 1985). For species with textural problems due to the TMAO breakdown, the storage temperature must be below −30°C.

84.11.5 Effects of Fluctuation in Storage Temperature

Fluctuations in storage temperatures affect the shelf-life stability of frozen products due to an increase in the size of the ice crystals formed in fish tissues (Shenouda 1980). With slight increases in temperature, small ice crystals melt faster than larger ones, so that when the temperature drops again, the melted ice refreezes around the large ice crystals, forming larger crystals. These large crystals accelerate freezing damage, leading to shorter storage stability.

84.11.6 Effect of Use of Additives

The effectiveness of different food-grade additives has also received considerable attention recently. The most commonly used types of additives for fish and seafoods function either as antimicrobial agents or as antioxidants. The selection of an antimicrobial agent or any combination of agents is rather complicated, especially when dealing with fish. The effectiveness of an antimicrobial agent depends on several factors, such as the moisture content of the product and the presence of other microbial inhibitors like smoke and salt. Several antimicrobial agents have been tested for fishery products. For instance, the sorbic acid salt, potassium sorbate (KS), has been found useful in extending the shelf-life of fresh fish. Studies have demonstrated that KS, when applied as part of the ice, increased ice storage stability of red hake and salmon up to 28 and 24 days, respectively (Fey 1980; Fey and Regenstein 1982). KS, in combination with modified atmosphere packaging (MAP), was also determined to be an effective method for prolonging the shelf-life of fresh whole and filleted haddock on ice (Regenstein 1982). The shelf life of fresh fish may also be extended under refrigerated conditions with the use of Fish Plus, which exhibits its preservative effect due to the combined action of components such as citric acid, polyphosphates, and potassium sorbate. Citric acid lowers the muscle pH, which consequently creates an optimum environment for potassium sorbate

to exhibit its antimicrobial effects. Dipping in Fish Plus has been found to extend the shelf life of lingcod on ice to as much as a week (Nawar 1996). Fish Plus may also be used on frozen fish.

An antioxidant is a substance capable of delaying or retarding the development of rancidity or other flavor deterioration due to oxidation. It is normally used in conjunction with freezing to reduce the rate of autoxidation during frozen storage. Antioxidants delay the development of rancidity either by interfering with the initiation step of the free radical reaction or by interrupting the propagation of the free radical chain reaction (Uri 1960). The kinetic of antioxidative action was considered to be that antioxidants act as hydrogen donors or free radical acceptors (AH) and react primarily with ROO· and not with R· radicals: $ROO\cdot + AH \rightarrow ROOH + A\cdot$.

A low concentration of this chain-breaking antioxidant (AH) can interfere with either chain or initiation, producing nonradical products:

$$\left.\begin{array}{l} A\cdot + ROO\cdot \longrightarrow \\ \\ A\cdot + A\cdot \longrightarrow \end{array}\right\} \text{nonradical products}$$

Various authors have suggested different versions of the antioxidant mechanism (Uri 1960; Hultin 1985). Other antioxidants may function as metal complexing agents, which partly deactivate the trace metals, often present as salts of fatty acid (Hultin 1985), which would otherwise promote the oxidative reaction. Citric, phosphoric, ascorbic, and erythorbic (isoascorbic) acids are typical metal-chelating agents. Among these antioxidants, erythorbic acid was used in studies by Licciardello and others (1980) and Kelleher and others (1981) of shelf-life stability of frozen fish. This antioxidant was emphasized due to encouraging results with the use of its salt, sodium erythorbate, in retarding oxidation in whiting, chub mackerel and white bass fillets (Greig 1967a,b; Licciardello and others 1980). Licciardello and others (1980) demonstrated the effectiveness of erythorbic acid in the retardation of oxidative rancidity in fillet blocks of Argentine hake stored at $-18^{\circ}C$. However, the use of erythorbic acid is limited to fish species in which rancidity is the main problem. Kelleher and others (1981) demonstrated the effect of this compound on the frozen storage of red hake (*Urophysis chuss*), a gadoid species, in which lipid oxidation is not the limiting factor for shelf-life extension. They found that the rate of DMA formation at $-18^{\circ}C$ in samples dipped in erythorbate solution was significantly greatly than the rate in untreated samples. Such effect of erythorbic acid on DMA formation may be explained by the fact that this acid acts as an alternative and preferred scavenger of oxygen. This leaves metal ions that would otherwise bind to oxygen and be inactivated, available to catalyze the degradation of TMAO to DMA and FA (Pedrosa-Menabrito and Regenstein 1988).

84.11.7 Effects of Packaging

Product preparation and packaging significantly affect the quality and shelf-life of frozen fish. If not properly controlled, these processes result in some deleterious effects after prolonged storage.

An efficient packaging system is essential to offset the detrimental quality changes that occur during frozen storage. Packaging materials and methods are obviously designed not

only to protect the product from microbial and chemical contamination, dehydration, and physical damage, but also to protect the environment from the packaged product. Fish and seafood can leak gases or unsightly fluids, which may have unpleasant odors. Therefore, the choice of appropriate packaging materials and methods for frozen fish is a critical factor in terms of shelf-life extension.

Studies have shown that packaging systems affect the quality and shelf stability of frozen fish. For instance, vacuum packaging is well established as a method to provide an oxygen-free environment to minimize the problems associated with lipid oxidation and dehydration during frozen storage. Several studies have shown the effectiveness of this method for frozen storage of some species of fish. For example, it has been reported that frozen blocks of fillets vacuum packed in moisture-proof films showed high degrees of acceptance and desirable frozen characteristics (Crawford and others 1979). Likewise, Santos and Regenstein (1990) reported the effectiveness of vacuum packaging for inhibiting lipid oxidation in frozen mackerel fillets. Ahvenainen and Malkki (1985) examined the influence of packaging on frozen herring fillets stored at different temperatures. They found that vacuum-packed product covered with metallized cardboard had a longer shelf-life than a product vacuum packed and stored without cardboard.

Vacuum packaging, on the other hand, need not be used if lipid oxidation is not the limiting factor affecting the shelf-life of a product. Although the effect or absence of oxygen in packages on some fish species must be considered, other packaging methods such as glazing and the use of heat-sealable packaging films should also be considered. Pacific hake minced blocks stored in moisture-proof, vapor-proof packaging films exhibited superior quality over glazed samples (Crawford and others 1979). Likewise, Colokoglu and Kundacki (1983) observed frozen mullet packed in plastic films with low permeability to oxygen and moisture to have a longer shelf life than when unpacked in the glazed form. However, it should be noted that glazing is still considered to be the cheapest means of protecting frozen fish during storage and transport. Glazing provides a continuous film or coating that adheres to the frozen product, which retards moisture loss and the rate of oxidation.

Many different glazes are available, including: (1) those with inorganic salt solutions of disodium acid phosphate, sodium carbonate, and calcium lactate; (2) alginate solution, otherwise known as the "Protan" glaze; (3) antioxidants such as ascorbic and citric acids, glutamic acid, and monosodium glutamate; and (4) other edible coatings such as corn syrup solids (Wheaton and Lawson 1985). Ice glaze is particularly important in handling frozen fish in developing countries. For products intended for short-term storage, glazing can be practically utilized as a viable alternative to storage without a protective covering. For instance, Jadhav and Magar (1970) concluded that glazing was a cheaper alternative to expensive packaging systems for glazed Indian mackerel (*Rastrelliger kanagurta*) stored at -20°C. Glazed samples had a shelf life of 6 months, while samples without a protective covering lasted only 3–4 months.

84.12 CONCLUSION

In summary, freezing, frozen storage, and thawing affect the quality and shelf stability of fish and seafood. If kept under appropriate conditions, fish and seafood can be stored in the frozen state for several months without appreciable changes in quality. During frozen

storage, microbiological changes in fish and seafood are very minimal. On the other hand, a series of changes such as protein denaturation, lipid oxidation, texture deterioration, loss of fresh odor and flavor, various enzymatically induced reactions, loss of volatile constituents, nutritional losses, and changes in moisture take place in fish and seafood when subjected to excessively prolonged frozen storage. Likewise, such changes may also occur in freeze-thawed fish and seafood. Quantitative evaluation of the influence of freezing, frozen storage, and thawing on fish and seafood is rather complex. The different variables that influence quality are related to one another. Therefore, it becomes almost impossible to describe some quality changes without actually discussing the other related changes that occur in fish tissues.

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85

Shellfish Freezing

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85.1 Introduction 894
85.2 Shellfish Varieties and Harvesting Methods 895
85.2.1 Shrimps 895
85.2.2 Lobsters 896
85.2.3 Crabs 897
85.2.4 Oysters 898
85.2.5 Clams 898
85.3 Freezing Methods 899
85.3.1 Sharp Freezing 899
85.3.2 Plate Freezing 899
85.3.3 Blast Freezing 899
85.3.4 Cryogenic Freezing 900
85.3.4.1 Carbon Dioxide Freezing 900
85.3.4.2 Liquid Nitrogen Freezing 901
85.3.5 Immersion Freezing 901
85.3.6 Pressure Shift Freezing 902
85.4 Glazing 902
85.5 Thawing 902
85.6 Packaging 903
85.6.1 Plastic Packaging Materials 903
85.6.2 Paper and Card Packaging Materials 904
85.6.3 Metal Packaging Materials 904

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

85.7 Physical Properties as Affected by Freezing Treatment 904
85.7.1 Changes in Odor and Flavor 904
85.7.2 Color Change 905
85.7.3 Free Liquor or Drip 906
85.7.4 Texture Changes 906
85.8 Microbiological Quality 907
85.9 By-Products of Frozen Shellfish 908
85.10 Quality Control and Assurance 909
References 912

85.1 INTRODUCTION

Freezing is the most adopted method of preserving shellfish. Since chilling is not sufficient for maintaining the shellfish quality for longer storage periods, freezing has developed to be an excellent storage method. Good freezing and cold storage enables the shellfish to be kept for very long periods. Spoilage of shellfish occurs due to putrefactive bacteria that produce degrading enzymes leading to autodigestion. The growth of spoilage bacteria and the enzyme activity can be reduced by lowering the storage temperature.

The method of freezing is the most influencing factor affecting the quality of the frozen product. The choice of a particular freezing system largely depends on the type of product to be frozen, the freezing time required by a particular product, the product conditions and the amount of raw material. A number of freezers or freezing methods are in practice in the industry and careful selection of freezing equipment is necessary for best quality products. The popular methods of freezing are: blast freezing, cryogenic freezing, liquid nitrogen freezing, carbon dioxide freezing, spray or immersion freezing, and pressure shift freezing. Each method has its own advantages or drawbacks over other methods.

During freezing and cold storage, products undergo a complex sequence of chemical, physical, bacteriological and histological changes. These changes become more apparent when the product is thawed. Color, flavor, and texture of the products are the most vulnerable characteristics, which are affected by freezing. In shrimps, crabs, and lobsters, formation of black or blue pigments is observed. Protein denaturation causes loss of juiciness and results in a poor texture of the product. Similarly, the pleasant taste of shellfish is lost during storage due to lipid oxidation. These problems can be overcome by providing the most appropriate freezing and subsequent thawing conditions.

Freezing alone is not sufficient for preservation and is merely a method for preparing the shellfish for storage at a suitably low temperature. Different types of pretreatments such as cooking, boiling, blanching, and precooling are performed before subjecting the shellfish to freezing method. The methods or steps for preparation are solely dependent on type of shellfish and its anatomy. For example, shrimps are generally blanched in salt solution prior to freezing and lobsters need to be cooked in brine solution. Similarly butchered crabs are cooked before freezing and oysters are frozen with or without shell. A brief description of each method and its suitability is discussed in the subsequent section of this chapter. To ensure understanding of different types of shellfish and their handling prior to freezing, the following section includes description about shellfish in general.

85.2 SHELLFISH VARIETIES AND HARVESTING METHODS

85.2.1 Shrimps

There are almost 300 species of saltwater and aquaculture shrimps that are marketed worldwide. Saltwater shrimps are categorized as warm and cold water species. Warm water shrimps are classified by shell color such as white, pink, and brown and harvested in the South Atlantic and the Gulf of Mexico. These are *Penaeus setiferus*, *P. aztecus*, and *P. brasileinsis* (http://www.ncfisheries.net/kids/3shrimp.htm). Cold water shrimps that possess firmer meat and sweeter flavor are caught in North Atlantic and Northern Pacific regions. These are *Pandalus borealis*, *P. dispar*, *P. goniurus*, *P. platyceros*, and *Cragon franciscorum augustimana*. The sea shrimps are captured by a trawl net from the stern of the trawler. The trawl is a large funnel shaped bag held open by otter boards for entrapping the shrimps. Since the trawl catches other animals too, considerable time is needed to separate the shrimps from other sea fishes (http://www.seafoodhandbook.com/harvest.html).

Aquaculture shrimps, especially black tiger shrimps (*Penaeous monodon*), are commonly raised in Asia. In 1996, the world shrimp production was around 3 million tons. According to FAO, Thailand is the top farmed shrimp producer while China and India produce 80,000 and 70,000 tons, respectively (http://www.fao.org/waicent/ois/press_ne/presseng/1997/pren9735.html). Another species indigenous to Indo-pacific region is the blue-legged or giant–long-shrimp (*Macrobrachium rosenbergii*). This shrimp species can be sorted into eight categories based on physical parameters into large, medium, small short-claw males, long-claw males, females without eggs and females with eggs, soft shell or newly moulted males, and terminal growth males (Lin and Boonyaratpalin 1988). The common gears used for harvesting these varieties are cast net, haul net, large dip net (George 1969), and sometimes electric catching gear (Liao and Chao 1982). Two harvesting techniques that are applied are referred as cull (continuous) and drain (batch) harvesting. In cull harvesting, the shrimp are captured by a single seine operation where five to six labors at different locations in the pond beat the water surface and walk in the same direction as the seine is being pulled. The advantage of this practice is that the small-sized shrimps are returned to the pond. In batch technique, the water is drained through a water gate behind which a bag net is fixed. Shrimps swept along the stream of water are collected in this net. Drain operation is usually applied when the shrimps attain market size or when the cessation of farming activities is forced by lack of water or fall of temperature (New 1988). Those live shrimps are immobilized and killed by dipping in iced water or ice brine (3% salt) slurry and then packed single-layered on ice. This layering prevents the shrimps from damaging one another and retards tissue degradation (New and Singkolka 1982).

Shrimps are low in fat and calories but are higher in cholesterol than most seafood (Gordon and Martin 1982) (Table 85.1). Fresh shrimps have a clean smell, with no trace of ammonia and indole. If caught many miles from shore, the shrimps are usually beheaded before the vessel reaches the dockside so that considerable space is saved.

After unloading from the vessel, ice is removed and the shrimps are conveyed to a rotary drum that removes the surplus water and debris. After this operation, the shrimps are weighed and graded according to size. For peeled and deveined products, the shrimps with shells on are peeled and deveined by hand or by a mechanized process. After peeling, the tail meat is washed and inspected. They may be blanched in salt solution

TABLE 85.1 Nutritional Value of Shellfish (per 100 g of Edible Portion)[1,2,3,4].

Items	Dungness Crab	Shrimp	Clams	Lobsters	Oysters
Calories	94[1]	84[1]	64[1]	80[3]	90[3]
Total fat (g)	1.1[1]	0.9[1]	0.8[1]	1[3]	3.2[3]
Saturated fat (g)	0.1[1]	0.3[1]	0.1[1]	0.6–1.9[4]	
Monounsaturated fat (g)	0.2[1]	0.2[1]	0.1[1]		
Polyunsaturated fat (g)	0.4[1]	0.4[1]	0.3[1]		
Dietary fiber (g)	0[1]	0[1]	0[1]		
Protein (g)	19[1]	18[1]	11[1]	16.2–21.6[4]	13.1[3]
Carbohydrate (g)	1[1]	0[1]	2[1]	0.8[4]	
Cholesterol (mg)	65[1]	166[1]	30[1]	60[3]	30[3]
Sodium (mg)	322[1]	191[1]	49[1]	17[3]	68.9–143.4[2]
Vitamin A (μg)	–	18–22[2]	–	60[3]	75[2]
Niacin (mg)	3.1[1]	1.58[2]			2.01[2]
Thiamin (mg)	0.47[2]	0.034[2]			0.067[2]
Riboflavin (mg)	0.167[2]	0.034[2]			0.233[2]
Vitamin B12 (mg)	8.9[1]	1.3[1]	43[1]		
Ascorbic acid (μg)		275–324[2]		17[3]	565[2]
Vitamin E (mg)				60[3]	1.05[2]
Copper (mg)	0.6[1]	193.2[2]	0.3[1]		869.4–1220.2[2]
Phosphorus (mg)	149[1]	–	147[1]		
Selenium (mg)	41[1]	34[1]	21[1]		
Zinc (mg)	4.7[1]	0.93[2]	–		11.89–14.97[2]
Iron (mg)	0.35[2]	2.6[1]	12[1]		5.45–8.20[2]
Manganese (mg)	16.9–20.9[2]	33.6[2]	0.4[1]		20.8–23.8[2]

Source: [1]http://www.wholehealthmd.com/refshelf/foods_view/0,1523,167,00.html.
[2]Gordon and Martin (1982).
[3]http://www.charlestonseafood.com/seafoodnutrition.htm.
[4]Wheaton and Lawson (1985a).

for about 10 min and dried to remove excess water prior to freezing. Four common forms of shrimps are prepared for the frozen markets. They are frozen headless, frozen peeled and deveined, uncooked frozen and breaded, and headed and unshelled (http://www.seafoodhandbook.com/harvest/shrimpforms.html).

A temperature of -32 to $-40^{\circ}C$ is recommended for freezing shrimps as at low temperatures, the development of rancid flavor is minimized (Dassow 1968). Such lower temperatures could be achievable by using blast or multiple freezers.

85.2.2 Lobsters

Lobster, the king of crustaceans, provides a sweet and succulent meat. Live lobsters have hard and intact shell and when the lobster is lifted, its tail curls (http://www.seafoodhandbook.com/safety/quality.html). Two species of lobster that are common in the market are the American lobster and rock lobster. American lobster or true lobster and Northern lobster (*Homerus americanus*) are caught from the main coast while rock lobster or spiny lobster (*Jasus lalandii*) are found off Florida West Coast, Southern California, and the Pacific, usually from Australia and New Zealand. The difference between those species is that the rock lobsters lack large claws but have long spiny antennae or feelers. Three rock lobster species that are marketed worldwide are *Panulirus*

argus, *P. interruptus*, and *P. Cyanus*. The lobsters are caught by either baited trap or trawlers. The trap that consists of an oblong box made of laths or wood slats spaced to allow the undersized animals to escape, is used to gather the inshore lobster. The latter gear is suitable for deep-sea lobsters inhabiting in water up to 200 fathoms deep.

Prior to freezing, different methods are used, for example, it needs to be cooked in 3% brine for 10–20 min. However, the heat requirement of deep sea lobster is only to cook the meat next to shell only. This is done to avoid sticking of the meat to the shell or it might give an electric shock. For spiny lobster, the preparation steps include breaking the tail from the body for removing the intestine.

Lobster meat is packed in cans for freezing with a normal packaging size between 14–16 oz. When lobster meat is stored frozen at a temperature of $-23^{\circ}C$ or lower, its quality is good and has an intended shelf life of 3 months.

85.2.3 Crabs

Crabs are found in the Atlantic and the Pacific and are divided basically into two categories such as the swimming crabs and the walking crabs. The dominant varieties in the market are blue crab (*Callinectes sapidus*), stone crab (*Menippe mercenaria*), dungeness crab (*Cancer magister*), Alaska king crab (*Paralithodes camschatica*) and tanner crab (*Chionectes* spp.). The crabs are mostly captured by single pots. This harvesting method is very selective and the products are of good quality. The pots are baited with fresh fish and dropped to the bottom of the ocean with a heavy line and marker buoy. The legal size male crabs are daily collected by hauling the pot up from the water. Trotline and dredges are other gears which are used for harvesting the blue crabs. The trotline, in which the baits or lures are dragged behind a vessel as it moves through water, is used to catch the crabs when the animals are actively feeding. The crabs in a dip-net are collected while the baits are raised to the water surface. The dredge, which has teeth along the bottom bar of metal frame to dislodge the animals from the bottom, is principally applied during winter. Two dredges are dragged from a boat and hauled alternatively (http://www.seafoodhandbook.com/harvest.html). The crabs are usually butchered by stationary iron blade and carapaces removed, after unloading. The animals are split into halves and cleaned. Both whole crabs and butchered crabs have to be cooked prior to freezing. The cooking is performed by subjecting the crabs in boiling water or 3% brine at temperature of $100^{\circ}C$ for 20–25 min, depending on species and form of preparation (Dassow 1968). In case of blue crabs, cooking retort may be applied at a temperature of $121^{\circ}C$ for 3–20 min. Meat removal of king crabs is done by shaking or blowing with water under pressure and sometimes with rubber rollers. In blue and dungeness crab, meat is removed manually.

Crabmeat is delicately sweet, firm but flaky and is categorized as white body meat and claw meat. The white meat includes lump, backfin and flake, or regular. Lump meat is the finest and most expensive. It consists of large, choice chunks of body meat. Backfin is smaller pieces of the body meat and flake is white meat from any part of the body (http://www.seafoodhandbook.com/harvest/crabmeatforms.html). Cut meat pieces are packed into cartons or trays for freezing. The meat in blocks of cartons is given an ice glaze before packing and shipped to cold storage having temperature between $-18^{\circ}C$ to $-23^{\circ}C$.

85.2.4 Oysters

Oysters differ from other crustacean shellfish due to higher carbohydrate and lower total nitrogen content in their flesh (Wheaton and Lawson 1985a; Jay 1992) (Table 85.1). Species such as Eastern oyster (*Crassostrea virginica*), Pacific oyster (*Crassostrea gigas*), and Olympia oyster (*Ostrea lurida*), are of economic importance. Oysters can be harvested by picking, tonging, and dredging. Picking is generally confined to the area exposed due to low tide. Tonging and dredging are common techniques adopted to capture the oysters as they collect the animals in larger numbers. Tongs consist of two poles crossed like scissors and possess toothed iron baskets at the end of the poles. They are lowered to the seafloor to scoop up the animals and then are closed before raising the tongs from the bottom (Wheaton and Lawson 1985b). Similar to the former gear, a dredge is a metal rake that is dragged across the bottom of the ocean, scraping up oysters in its path. The oysters are carried up from the bottom either by a conveyor belt or flowing water through a large diameter hose (Banks and others 1977).

If the oysters are marketed in the shelled form (unshucked), only washing, packing, and chilling is required. Though most oysters, however, are sold as shucked meat, which is prepared by manual labor. After shucking, oysters are preliminary rinsed as the pieces of shell and torn or discolored and culled out. Then they are aerated in blowing tanks to remove sand, silt, and shell fragments. The washed meat is graded by size and packed into suitable packaging (Wheaton and Lawson 1985c), which is dependent on the freezing method. For example, compression plate freezing are suitable for meat packed in waxed cartons and overwrapped while blast tunnels are suitable for meat packed in cans. Freezing rates should be high and freezing temperatures should be as low as possible. Recommended temperature of freezing is -18°C, although oyster meat may remain in good condition for more than 9 months when stored at -29°C (Peter 1968). However, storing oyster meat for more than 6 months degrades the raw material quality, especially if the storage condition is not easily consistent.

85.2.5 Clams

Clams may be hard-shelled or soft-shelled and the edible portion consists of the muscles, the siphon, and the feet. Clams are generally sweet and a bit chewy. Several varieties are available in the market and their flavor and tenderness depend on the size and species. Some of them are hard clam or quahog (*Venus mercenaria*), sea clam or skimmer clam, or surf clam (*Spisulla solidissima*) and soft clam (*Mya arenaria*). These bivalves are scooped from the sand at low tide and from beds in deeper water on Atlantic and Pacific coast by dredging operation. Live clams after capture should be tightly closed, with a fresh smell. The neck of soft shelled clams retards when touched (http://www.seafoodhandbook.com/safety/quality.html). After harvesting, the clams are first washed to remove sand and silt and the shell is then removed. In some processes, the clams are eviscerated by squeezing the meat that removes the stomach and other soft tissues. The shucked animal should not dry out, shrivel or discolor or its quality is degraded substantially. Clams may be chopped, sliced into strips, or left whole before packing and freezing. Like other shellfish, storage and freezing type are also dependent on the type of container used. Smaller packages (2 kg) are best frozen by compression plate method and larger cans are recommended to freeze by either blast or shelf coil methods.

85.3 FREEZING METHODS

Freezing and subsequent cold storage is an excellent process for preserving the quality of shellfish as long as 18 months or more. During this period, a complex series of chemical, physical, bacteriological, and histological changes takes place. The factors that must be considered while adopting a freezing or storing technique are listed below (Banks and others 1977).

- Type of product to be frozen;
- Allowable freezing time for products of average weight;
- Handling requirements;
- Source of available power;
- Space requirement;
- Amount of raw material;
- Cost of equipment and operation.

85.3.1 Sharp Freezing

Sharp freezing is generally considered as slow freezing method because it takes 3–72 h, depending on the quantity of the product to be frozen. The procedure consists of placing the products in a cold room where temperatures are maintained in the range of $-20^{\circ}C$ to $-40^{\circ}C$. The disadvantage of this method is its low freezing rate and high labor cost. In addition, the cooling coils may frost during the loading and unloading of the products. Therefore, defrosting is required at least once every 6 months. Sharp freezer consists of an insulated room with multishelf racks for holding the product, which are stacked one above another. Each shelf is constructed of pipe formed into a horizontal flat coil. Refrigerant, especially ammonia is expanded through the coils lowering its temperature to the desired level.

85.3.2 Plate Freezing

Plate freezer is accomplished by direct contact between a cold plate and the products. Pressure applied to the plates on each side of the products improves contact and increases the heat transfer coefficient between the plate and the products. The pressures applied by an hydraulic press, in the range of 1 and 10 bar. Since the pressure exerted is constant expansion takes place as the product freezes. Consequently expansion takes place inside the package until all voids are filled. Approximately 7% expansion occurring in the products during freezing is sufficient to fill voids and compress the products into single block during freezing. The two most common types of plate freezers are the horizontal plate freezer and vertical plate freezer, both made from extruded aluminium. Both types efficiently freeze only regular-shaped packages or blocks. Horizontal-plate freezers are likely used to freeze prepacked retail flat cartons of shrimps, both with and without shell. The products are usually wrapped in plastic film and then packed in cartons or directly on to aluminum freezing trays, which are in turn, placed on the freezer shelves (Garthwaite 1997). Plate freezing is also applied to spiny lobsters and clams.

85.3.3 Blast Freezing

Blast freezing is a method where cold air having a temperature between $0–40^{\circ}F$ is used to remove the heat from the products before transporting it to the refrigeration coils.

Although the general operating principles, there are several types of blast freezers, varying in airflow, loading method, and capacities. Most blast freezers use average air velocities of 2.5–7.5 m/s, while 2.5–5.0 m/s is reported as the most economical velocity range. Blast freezing is done either by tunnel freezing or fluidized bed freezing.

In tunnel freezing method, the products are placed on trays, either loose or packaged. The trays are placed on a moving mesh belt passing through a tunnel or enclosure where the cold air is blown from the opposite end. In some designs, cold air is circulated on both top and bottom ends of the entire length of the freezing belt so that a better distribution of the cold air is obtained. The main advantage of tunnel freezers is that they are versatile and are suitable for irregular-shaped, different-sized and nondeformable foods such as crustaceans, fish fillets, and added-value products. However, tunnel freezers have a slightly slower freezing rate compared with immersion freezing. Besides dehydration of the products may occur during the operation, which results in the constant need of defrosting the equipment. To reduce the moisture loss from the products, two or more stages of freezing are introduced. It is because when large volumes of air of high relative humidity are applied in the first stage, the products are frozen with minimum water loss. In the later stage, the temperature and vapor pressure differences are not so high. Therefore, the cold air has substantially less desiccating effect.

In some plants, where high capacity or extended freezing time is required, the use of spiral freezers is suggested. The single, continuous belt can be operated on a single- or twin-drum application in ascending or descending combinations. The whole system is enclosed in an insulated chamber. Blast freezing is suitable for many aquatic food products such as for king crab in the shell packed into tray or carton, whole dungeness crab in can and peeling and deveining shrimp on thin aluminum sheet. Due to high demand of IQF frozen products, fluidizing belt freezers are extensively used. The procedure requires a sufficiently powerful stream of cold air to keep the products in suspension. The advantages of fluidized bed freezing are short freezing time since each piece of food is kept loose and free flowing by the air pressure, resulting in higher yield. Retention time for freezing operation depends upon the size of the products, for example, small shrimps require 6–8 min as the large size shrimps require 12–15 min. Freeze-Pak fluidized belt freezer and Lewis fluidized bed freezer are the common type of fluidized freeze dries (Banks and others 1977).

85.3.4 Cryogenic Freezing

Cryogenic freezing is the ultra fast freezing process where the products are exposed to an extremely cold refrigerant. The products could be packed or thinly packed and the resulting quality of the products is very high. In this method, the heat removal is accomplished during a change of state by the refrigerant. The advantages of cryogenic freezing are rapid rate of freezing, simplicity, flexibility, and inexpensive equipment design. Refrigerants that are commonly used in plants are liquid nitrogen or carbon dioxide (Garthwaite 1997).

85.3.4.1 Carbon Dioxide Freezing. Carbondioxide exists in liquid state about its critical condition of $-31^{\circ}C$ and 7.35 MPa absolute pressure. Its triple point is at $-56^{\circ}C$ and 7.35 MPa absolute pressure. Freezing with carbon dioxide is done by passing the products under specially designed nozzles. Liquid CO_2 supplied to the nozzles at about 300 psi is sprayed toward the products as they move under the nozzles

on a conveyor belt. Due to the high pressure, the CO_2 changes its state as it leaves nozzles and absorbs large quantities of heat from the products.

The principle behind using carbon dioxide is that at atmospheric pressure and room temperature, solid CO_2 (dry ice) converts directly from a solid to gaseous state (sublimation) leaving no liquid residue. As sublimation of dry ice occurs at $-78.5^{\circ}C$, it is possible to freeze a product to a temperature as low as $-75^{\circ}C$ (Wheaton and Lawson 1985d). Freezing under these circumstances is very rapid and drip losses are reduced to less than 1% (Garthwaite 1997). A good example of using dry ice as cryogenic freezing is thermice process. The operation involves mixing of the comminuted dry ice with the products in the interior of a slowly rotating and insulated drum. The rotation of the cylinder turns the products over and over in dry ice and also break up the gas film on the product as well. It is noted that all of dry ice must be separated from the frozen products before the products are packed otherwise the package may explode due to the pressure of the gaseous CO_2 in headspace (Banks and others 1977).

85.3.4.2 Liquid Nitrogen Freezing. The critical point for nitrogen is much lower than that of carbondioxide, though it is achievable at $-147^{\circ}C$ and 3.39 MPa. The triple point occurs at $-210^{\circ}C$ and 12.6 kPa. Liquid nitrogen is nontoxic and relatively cheaper and is obtained as a by-product during production of oxygen from air. Liquid nitrogen freezing systems are divided into three types; that is, immersion type, spraying of liquid nitrogen and circulation of cold nitrogen vapor. However, the spray method is commonly applied in food industries in which the products are placed on a conveyor belt in a single layer moving in the opposite direction to the flow of nitrogen. Warm products entering the freezer are first subjected to a blast of cold nitrogen gas (typically at about $-50^{\circ}C$). This precooling prevents stress cracking in the products that can occur due to rapid cooling. Later, the products are headed to direct application of liquid nitrogen, which has a boiling temperature of $-196^{\circ}C$ at atmospheric pressure. In the last freezing section, equilibrium of temperature occurs between outside and the center of the products (Wheaton and Lawson 1985d).

The application of liquid nitrogen is commonly applied for shrimps and oysters. The frozen products have lower indole and trimethylamine contents (Bank and others 1977). In addition, the protein changes are less and the drip loss during thawing is low.

85.3.5 Immersion Freezing

Immersion freezing is a simple technique, in which the products are immersed into a low temperature liquid such as sodium chloride brine, sugar solution, or glycerol. Sodium chloride that has a eutectic point of $-212^{\circ}C$, is normally applied in the freezing process at a temperature of about $-15^{\circ}C$. Further reduction in temperature is achieved by transferring the products to cold storage. The limitation of immersion freezing is the suitability of refrigerating medium (Garthwaite 1997). The freezing medium should contain chemicals that should be edible and be capable of remaining unfrozen at $-17.8^{\circ}C$ and slightly below. The refrigerating temperature also needs to be carefully controlled. If the medium temperature is too high, it will enter the products by osmosis and if too low, the medium may freeze the products. Moreover, it is difficult to maintain the concentration of medium at a fixed value (Banks and others 1977). Crabs and shrimps are commonly frozen by brine immersion. Cooked whole and eviscerated crabs in shell are dipped into circulating brine of 88° salometer

at −18 to −15°C for 45 min then brought into fresh cold water to remove excess brine and provide an ice glaze (Garthwaite 1997).

85.3.6 Pressure Shift Freezing

Pressure shift freezing is carried out in a high pressure vessel where the temperature is controlled at subzero levels. The advantage of this technology is that it achieves uniform super-cooling in the products. The products are at first refrigerated under pressure and no ice crystals are formed in this step. The pressure is then released to atmospheric pressure. This phenomenon causes three significant phases. The first phase corresponds to cooling down the products without phase change. In the second phase, a sudden temperature rises up to the phase change temperature at the current pressure. Finally, partial freezing is initiated due to the high super-cooling of the product (Chevalier and others 2000).

85.4 GLAZING

Glazing is the process of coating a frozen product with a layer of ice to retard the process of moisture loss and oxidation. Glazing is usually accomplished by dipping the frozen products into a tank of chilled water or by spraying a light coating of chilled water onto the frozen products. The low product temperature results in the formation of a layer of ice on the exterior of the product. The amount of ice per product varies based on the temperature of the frozen product and glazing water, and the residence time in the glazing tank. Dipping in tank could lead to the possibility of contamination, especially if the water is not so clean and therefore spray system provides an added advantage over the dipping method. Different additives are applied in chilled water for different purposes (Santos-Yap 1996). The most common additives are organic salt solution of disodium acid phosphate, sodium carbonate and calcium lactate; alginate solution; ascorbic, citric and glutamic acid and monosodium glutamate and other edible coatings such as corn syrup solids.

The additives help in preventing oxidation, improving the appearance of the products, and strengthening the ice layer. This prevents the product to become brittle and fracture when it is bumped or dropped (Pigott and Tucker 1990).

Glazed products have a shelf life of at least 6 months while products without a protective covering last only 3–4 months. During storage evaporation occurs from the glazed layer only. Other preferable technique is to subject the product to a moisture-vapor proof over-wrap on the product package. This ensures prevention of water loss during prolonged storage under freezing condition.

85.5 THAWING

Appropriate method and conditions of thawing are as important as selecting a suitable freezing method because it can affect the net weight of the products. Improper thawing under forced conditions of warm air or water may cause the products to release its natural juice, thus drying out the product and aid bacterial growth. Therefore, the frozen products should be thawed slowly at temperatures just above freezing

(http://www.seafoodhandbook.com/harvest/frozen.html). Special equipment has been devised for the purpose of thawing, so that the damage to the quality of the product is minimal. During thawing, the frozen blocks are subjected to dielectric heating, being conveyed on a rubber belt through a series of dielectric units. To get an even heat flow across the frozen pieces, blocks are first immersed in plastic trays of water to fill up the voids in the blocks. It takes one hour to thaw a 4-inch thick block (Dassow 1968). Alternatively, cross-flow air blast devices are also used to thaw the frozen blocks.

85.6 PACKAGING

Packaging plays a significant role in minimizing quality loss within the products during periods of frozen storage. The suitable packaging should provide an effective barrier to the ingress of moisture, gasses, and any contaminants from the environment to the products. The special requirements for frozen shellfish packaging are temperature stability and insulation properties. The package material should be physically as well as chemically stable over a wide range of temperatures. It should withstand each stage of production, distribution, and storage. Insulated packages may help to maintain a low temperature throughout the bulk of products during periods outside the freezer and to minimize temperature fluctuations within food products. The common materials used for the packaging of frozen shellfish are plastic materials, metal and paper.

85.6.1 Plastic Packaging Materials

One of the most important characteristic of packaging materials for frozen foods is the low permeability to water vapor. Table 85.2 demonstrates the properties of plastic materials which are commonly used. Low density and high density polyethylene (LD- and HDPE) provide relatively good barrier protection to water vapor but poor barrier protection from O_2 and CO_2. HDPE and polyester terephthalate (PET) can withstand high temperature so they are often used in the prepared foods market. Although polystyrene (PS) has relatively poor barrier characteristics to both water vapor and gases, it is a good thermal insulator and has high impact resistance at freezer temperature. Therefore, it is generally used as a secondary package material (George 2000). Laminate and coextrusion materials built up from two or more of these materials or other films are also used to gain the specific properties required.

TABLE 85.2 Permeability Coefficients ([cm^3][cm]/[cm^2][s][Pa]) of Water and Gases in Plastic Films.

Film Type	Temperature (°C)	Permeability Coefficient × 10^{13}			
		Water Vapor	O_2	N_2	CO_2
Polyethylene, LD	25	93	5.18	1.58	21
Polyethylene, HD	25	13.5	0.825	0.248	3.225
Polypropylene	30	51	1.7	0.33	6.9
Polystyrene	25	1350	1.9	–	–
Polyvinylchloride	25	206	0.034	0.0089	0.12
Polyvinylidene-chloride	30	7.0 (at 25 °C)	0.000383	0.000706	0.0218

Source: Piringer (2000).

The film materials can be used as wrappers (over-wrap and shrink-wrap), bags and liners while semirigid plastics are usually manufactured in the forms of trays and plates, covered by lids.

Recently, modified atmospheric technique has been introduced to improve the retention of quality of frozen food. This is by retarding microbial, physical, chemical, and biochemical changes associated with deteriorative process (Bak and others 1999). Other innovative form is intelligent or smart packaging. Temperature and time-temperature indicators attached to the package are designed to monitor the history of the products (Selman 1995). Other special devices are gas sensing dye, microbial growth indicators, and physical shock indicators.

85.6.2 Paper and Card Packaging Materials

The packages from these materials are in the form of both folded and rigid cartons. The corrugated fiberboard or vulcanized fiberboard paper are used as the shipping container. The materials are usually coated or laminated with wax blend (paraffin and microcrystalline composition) or plastic (polyethylene or polypropylene) to provide adequate barrier protection (Jiang and Lee 2004).

85.6.3 Metal Packaging Materials

The metal commonly used in frozen food packaging is steel and aluminum. Both of them provide highly effective barrier properties for water vapor, oxygen, and light. As cited by George (2000), aluminum becomes more attractive because it can be used safely within microwave ovens. The frozen foods in form of ready meals or ready to cook products are usually packed in aluminum trays, dishes, or cups which offer good rate of heat transfer. These packages are covered with crimped-on aluminum sheet or a sheet of aluminum foil laminated to paperboard (Jiang and Lee 2004).

85.7 PHYSICAL PROPERTIES AS AFFECTED BY FREEZING TREATMENT

Changes in physical characteristics of products such as color, flavor, and texture occur immediately after harvest. If the products are not properly handled during freezing, frozen storage, and thawing, they undergo quality changes hence making the products unacceptable to market and consumers. When the product is to be exported to European or American markets, then the quality standards are more critical. The most common quality parameters that affect the marketability, the price and acceptability of shellfish products are described in the following section.

85.7.1 Changes in Odor and Flavor

Processing conditions of freezing, storing, thawing, and cooking have maximum effect on the flavor and odor of shellfish. Since the flavor and odor components are usually volatile, a slight change in the temperature of processing, be it any stage effects the taste of the shellfish, which is the most important criteria for consumer acceptance. Shellfish have a mild and sweet taste, that is lost quickly when stored under unsuitable conditions.

Generally odor changes occur in three phases, that is, gradual loss of flavor due to loss or decrease in concentration of some flavor compounds; the detection of neutral, bland, or flat flavor; and the development of off-flavor due to the presence of acids and carbonyl compounds from lipid oxidation and degradation of trimethylamine oxide (Santos-Yap 1996). Flavor and odor components found in shellfish are mostly classified as nitrogenous compounds. Those nitrogenous compounds comprise free amino acids, low-molecular weight peptides, nucleotides, and organics.

Shrimps and crabs possess high level of amino acids such as taurine, proline, glycine, alanine, and arginine but only traces of peptides are detected. Nucleotides serving as important palatable taste producing factor are found in shellfish as adenosine monophosphate (AMP). Small amount of adenosine diphosphate (ADP), inosine monophosphate (IMP), guanosine monophosphate (GMP), and uracil monophosphate (UMP) are detected in the leg meat extracts of boiled crab as well (Konosu and Yamaguchi 1982). Trimethylamine oxide (TMAO) is a common base usually found in muscle of fish and shellfish. In postmortem stage, this compound is reduced from trimethylamine which provides fish odor by bacterial strains of Enterobacteriaceae including, *Escherichia coli*, *Achromobacter*, *Micrococcus*, *Flavobacterium*, nonfluoresscent *Pseudomonas*, *Clostridium*, *Alcaligenes*, and *Bacillus* spp. (Regenstein and others 1982). As cited by Konosu and Yamaguchi (1982), TMAO is detected in crabs and shrimps in the range of 65–140 and 172–213 mg/100 g, respectively. The other important quaternary ammonium base is glycine betaine found in crabs and shrimps in amount of 357–711 mg/100 g and 251–961 mg/100 g, respectively. The variations depend upon species, growth, freshness, parts and tissue, season as well as environment condition. Other decomposed odor in shrimps is indole. This strong odor is a result of highly proteolytic, indole positive bacteria such as *Aeromonas* and *Proteus* spp. These microorganisms attack muscle protein and convert tryptophan to indole. The reaction is aggravated when the shellfish is stored under high temperatures therefore, high level of indole may be used to indicate temperature abuse (Smith and others 1984).

85.7.2 Color Change

Among all the shellfish, shrimps are more prone to color changes. The binding of protein with asthaxanthin, the dominant color component results in grayish color to shrimp. As soon as the shrimp is subject to heat, such as boiling, the attached protein degenerates, and pink color of the shrimp appears. Many a times, there is a rapid formation of black pigments which is referred to as melanosis, occurs within few hours after death and is enhanced by exposing the shrimps to air. This reaction is the result of phenol oxidation in the internal shell surface and can occur within 2–12 h of exposure to air (Banks and others 1977). The reaction can occur even at 0°C, however, at −18°C, no visible spots are detected for 3 months (Garthwaite 1997). The discoloration starts at both the ends of overlapping shell segments and then it develops into black bands or zebra appearance. In advanced stage, the oxidation reaction of tyrosinase on tyrosine results in melanin pigment on the underlying shrimp meat. Copper and other metallic irons can accelerate the reaction. Thawing at even 0°C could lead to melanosis due to the unnecessary exposure of the shrimps to air thus leading to oxidation.

In crabs and lobsters, too, the development of blue or black discoloration referred to as blueing is one of the most common and severe problems. Blueing may occur after freezing or during frozen storage, after thawing or even shortly after cooking. This bluish-black

curd-like discoloration appears to be related to biuret-type reaction, which occurs due to the copper pigments in the circulating fluid and the heat denatured protein. In crab as well, blueing relates with the change of phenolic compounds in crab as well (Babbitt 1982). Tyrosinase and phenol oxidase in live crabs initiate oxidation reaction followed by nonenzymatic oxidation and polymerization. The oxidation particularly occurs under alkaline conditions and or in the presence of metals such as copper and iron. Molting stage can aggravate the incidence since the phenolic compounds are involved in the formation of the new shell. Blueing discoloration in king crab meat could be reversed by using a reducing agent such as sodium sulfite solution (Banks and others 1977). Antioxidants such as ascorbic acid and metal chelating substances like phenylthiourea are also active in inhibiting the action of oxidases in crab (Babbitt 1982). Heating the crabs at 100°C for 20 min inactivates all enzyme activities. However, the best way to reduce the blueing discoloration is processing only live crabs that are properly harvested and handled.

In addition to blueing, yellowing, and fading of the red or orange-red carotenoids also occurs in crab and lobster. Both infer the degree of oxidation during processing or prolonged cold storage, which solely depends upon the retention time of exposure to air and temperature, freezing, and storage conditions. Microorganisms also cause discoloration in some shellfish, for example, Asporogenous yeast can grow and produce pink pigment when contaminated in oyster (Jay 1992).

85.7.3 Free Liquor or Drip

Free liquor or dripping occurs during thawing of the frozen shellfish products. The drip or exudate formation is directly related to the capacity of the animal protein to hold moisture (Garthwaite 1997). A cloudy liquid occurs due to the rupturing of cell walls caused by ice crystal formation during freezing. During cooking, there is an increase in the release of liquor resulting in the loss of water-soluble proteins, particularly sarcoplasmic protein, vitamins, and minerals. This indicates inappropriate handling, prolonged ice storage prior to freezing, improper cold storage or thawing technique. For example, frozen oyster could have a drip loss of over 20% depending on the conditions of blowing (Banks and others 1977).

85.7.4 Texture Changes

Texture is one of the most important parameters of cooked shell fish, though it varies with individual customer's acceptance. The textural changes are usually caused by protein denaturation. Frozen shellfish lose their juiciness and succulence after freezing and subsequent frozen storage. The muscle of the shellfish loses all its moisture at the first bite and this leads to a dry and slightly tough texture. Species and storage temperature are the main factors affecting the change in texture, for example; dungeness crab meat is less juicy than king crab meat when kept at −18°C. Lower storage temperature can also improve the storage quality of aquatic animals.

In some species of shellfish, mushiness or softening of the flesh occurs. These include sand crab (Slattery and others 1989), rock lobster (Wessels and Olley 1973), and blue legged shrimp (Baranowski and others 1984). This texture deterioration is due to the proteolysis of digestive enzymes from hepatopancreas (Nip and others 1985; Slattery and others 1989). Mushy shellfish is externally indistinguishable from the fresh animals and the condition becomes evident only after cooking. Poor handling can aggravate quality deterioration by accelerating the rate of degradation. Blanching can be used to lessen

the problem because enzymes are inactivated at temperatures higher than 70°C. Deheading is the other way to diminish the enzyme problem especially in shrimp. By doing this, the hepatopancreas, the major source of digestive enzyme, are removed. The disadvantage of deheading is that the product possesses less flavor because of the removal of the hepatopancreas which is the main source of flavor (Vongsawasdi and Noomhorm 2000). Moulting stage is also a crucial factor affecting the degree of mushiness. Pre- and postmoult shrimps are mushier due to a larger proportion of short fiber compared to the intermoult stage (Angel and others 1986; Slattery and others 1989). Moreover, the animal begins to absorb water upon entering the premoult stage. Such water may soften the tissue by disintegrating the interfiber connection within the muscle. After moulting, the amount of water in the tissues gradually decreases but it is enough to cause significant myofibrillar disruption and consequently mushiness occurs (Angel and others 1986).

85.8 MICROBIOLOGICAL QUALITY

Freshly caught shellfish are highly perishable due to accelerated bacterial activity at normal temperature. Due to this, the animals are either frozen or boiled immediately

TABLE 85.3 Microbial Spoilage of Some Shellfish.

Shellfish	Microorganisms
Shrimp	*Acinetobacter*[1] *Moraxella* (at 5–11°C)[1,2] *Vibrio*[1] *Pseudmonas* (at 0°C)[1,2] *Proteus* (at 16–22°C)[2]
Crab meat	*Pseudomonas*[1] *Acinetobacter*[1] *Moraxella*[1] *Proteus*[1]
Raw lobster	*Pseudomonas*[1] *Alcaligenes*[1] *Flavobacterium*[1] *Bacillus*[1] *Vibrio* (including *Vibrio parahaemolyticus*)[1]
Oyster	*Pseudomonas*[1,3] *Acinetobacter*[1,3] *Moraxella*[1, 3] *Serratia*[3] *Proteus*[3] *Clostridium*[3] *Bacillus*[3] *Escherichia*[3] *Enterobacter*[3] *Flavobacterium*[3] *Lactobacilli*[1,3] Yeasts[1,3]

Source: [1]Frazier and Westhoff (1988).
[2]Hayes (1992).
[3]Jay (1992).

after capture. The incidence and number of microorganisms greatly depend on the quality of water from which these animals are harvested. The initial flora found in freshly caught oysters are *Alcaligenes*, *Flavobacterium, Moraxella*, *Acinetobacter* spp., while shrimps, crabs, and lobsters have a bacteria laden slime on their body surfaces including *Bacillus*, *Micrococcus*, *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Flavobacterium*, *Alcaligenes*, and *Proteus* spp. (Frazier and Westhoff 1988). When the shellfish are frozen, these microorganisms are generally inactivated and microbiological changes in shellfish tissue are minimal. Although some microorganisms survive with their activity temporarily suppressed, bacterial numbers may be considerably reduced if recommended temperature is maintained. The temperature below which microbial growth is considered to be minimal ranges between -10 to $-12^{\circ}C$ (Matches 1982). However, the survival microorganisms, usually psychrotrophic bacteria, for example, *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, and *Flavobacterium* spp., may grow after thawing and lead to microbial spoilage of the thawed products (Frazier and Westhoff 1988; Hayes 1992) (Table 85.3). The microbial activity depends on the degree of freshness of the raw material, natural microflora in the shellfish tissues and the thawing technique adopted.

85.9 BY-PRODUCTS OF FROZEN SHELLFISH

The meat is the most important component of shellfish, however waste from these crustaceans could be extremely valuable too. The shellfish waste from shrimp, crab, squid, cuttlefish, oyster, clams, and lobsters is rich in chitin, a biopolymer with innumerable applications (Knorr 1984). The shellfish such as shrimps also consist high quantities of protein as well as minerals (Rao and Stevens 2005). In many countries the biowaste obtained from the crustacean processing factories is released in the environment resulting in severe pollution of soil and surface water. In some countries the biowaste is sold to the feed mills at low prices. Realizing the potential application of chitin and its derivatives, many companies utilize the waste by processing immediately for chitin production or sell to other companies at better prices, to be eventually converted to chitin. To preserve the quality of waste, it needs to be sold or processed in frozen conditions.

The chitin content in crustaceans is very high varying from 11% in shrimp heads, 27% in shrimp shells, 12–18% in commercial shrimp waste, 24% in snow crabs, and 30–35% in squid pens. Chitin is a naturally occurring polymer constituting of N-acetylglucosamine units, some of them being deacetylated (Sannan and others 1976). The deacetylated derivative of chitin is known as chitosan, which has many applications in different sectors. The monomer of chitin is 2-acetamido-2-deoxy-β-D-glucose and that of chitosan is 2-amino-2-deoxy-β-D-glucose.

The free amine group gives chitosan its primary properties useful in natural polymer application development. When dissolved in dilute acid, chitosan becomes a cationic polymer, linear in structure, with a high positive charge density. This electronic charge can then be used in flocculation process, film forming, or immobilization of various biological reagents including enzymes. Other chemical properties of chitosan include its ability to act as a moisturizing agent in cosmetics, chelation of multivalent cations and an encapsulation agent when positive charge is removed (Ornum 1992). Chitosan fibers or granules applied to skin seem to keep fungi and microorganisms from growing, and therefore it has found application in textile industry. It even appears to stimulate the growth of beneficial bacteria in the digestive tract (Pennisi 1993). These polymers are

further used in wound healing, blood clotting, cholesterol lowering drugs, and controlled release of drugs in the body.

Besides chitin and chitosan, the shellfish could be a source of high value protein and minerals. Protein and mineral hydrolysate could be obtained by Lactobacillus fermentation of shrimp waste, where lactic acid produced during fermentation dissolves the minerals and the proteases produced contribute to proteolysis (Rao and Stevens 2005). This dual phenomenon results in a liquor rich in protein and minerals with a strong shrimp flavor. The liquor could also be converted into powder or paste for further applications.

The specific applicability of chitin and chitosan and other derivatives depends on its various properties such as molecular weight, viscosity, degree of acetylation, transparency, particle size, and solubility. The properties of chitin and chitosan are affected by the source and quality of raw material. This value addition to shellfish indicates that appropriate storage is not only important for food applications, but also for utilization of the so-called waste product of the shellfish industry.

85.10 QUALITY CONTROL AND ASSURANCE

Quality control is the process concerned with sampling of the products and measurement of quality indices to ensure that the products meet the required standards. Quality assurance, which is different than quality control, emphasizes on the processes and procedures which assure food safety and quality. In addition, this system is less dependent upon end product testing.

The examples of chemical indices for quality control are K value, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), hypoxantine, and indole. Many researchers suggest that TVB-N and TMA are excellent indicators for determination of shellfish freshness (Fatima and Qadri 1985; Noomhorm and Vongsawasdi 1998; Shamshad and others 1990). Indole is considered a useful chemical index of shrimps however it should not be used solely as decomposed shrimps do not necessarily contain indole (Botta 1995).

The important element used as a means to assure food safety is Hazard Analysis and Critical Control Point (HACCP) which contains seven principles of identifying the potential hazards, determine the critical control points (CCPs) for the identified hazards, establish the target levels/tolerances for controlling the CCPs, establish/implement monitoring systems for controlling CCPs, identify corrective actions when a deviation occurs at a CCP, verify that the HACCP system is working and establish a documentation system for procedures and records (Pierson and Corlett 1992). The system must apply to the whole process, from raw materials to finished products otherwise an unacceptable food safety risk may occur. Generally, the procedures in developing the HACCP consist of two parts. The first part copes with hazard analysis which includes the determination of the critical limits and the identification of CCPs. Table 85.4 shows the hazard analysis of frozen shellfish. The possible hazards associated with the handling of fresh shellfish are physical damage, chemical contamination, microbiological contamination, autolysis, and other biochemical reactions. During freezing process and storage, there are further opportunities for physical, microbiological, and biochemical degradation as well. The second part of the procedure concerns with monitoring and verification. In this section, the CCPs can be defined as locations, procedures or processing steps at which hazards can be controlled. Table 85.5 represents a HACCP plan for frozen shellfish. Since the information from Table 85.4 indicates that metal inclusions may threat consumer

TABLE 85.4 Hazard Analysis of Frozen Shellfish.

(1) Processing Step	(2) Identify Potential Hazards Introduced Controlled, or Enhanced at this Step (1)	(3) Are any Potential Food-Safety Hazards Significant? (Yes/No)	(4) Justify Decisions for Column (3)	(5) What Preventive Measures can be Applied to Prevent Significant Hazards?	(6) Is this Step a Critical Control Point? (Yes/No)
Receiving	Biological: contaminated with pathogens, autolysis, biochemical reactions	Yes	Raw material is known source of pathogens Proper temperature control should help to reduce their level		No
	Parasites	No	Parasites are killed during extended frozen storage.		
	Chemical: sanitizers, disinfectants	No	Unlikely to occur. Controlled by SSOPs.		
	Physical: foreign objects	No	Low risk, Unlikely to occur. Controlled by SSOPs.		
Freezer	Biological: None Chemical: None Physical: None				
Weighing	Biological: None Chemical: None Physical: None				
Packaging	Biological: None Chemical: None Physical: None				
Metal detector	Biological: None	Yes	Any harmful physical foreign materials in the product may present hazard to consumers.		Yes
	Chemical: None Physical: foreign objects and metal inclusion				
Frozen storage	Biological: pathogen growth during storage	No	Unlikely to occur. Controlled by SSOPs.		
	Chemical: None Physical: None				
Shipping	Biological: pathogen growth during storage	No	Unlikely to occur. Controlled by SSOP.		
	Chemical: None Physical: None				

TABLE 85.5 HACCP Plan for Frozen Shellfish.

Critical Control Point (CCP)	Significant Hazard	Critical Limits for Each Preventive Measure	Monitoring				Corrective Action(s)	Record	Verification
			What	How	Frequency	Who			
Metal detector	Metal inclusion	No detectable metal fragments in finished products	Presence of metal fragments in finished products	Metal detector	Every finished product packages	Production employee	Destroy any products rejected by metal detector Identify source of metal and fix the problem Quarantine any products processed without metal detection for metal detection	Metal detector operation log	Test metal detector before beginning operation Review monitoring, corrective action every week

health, metal detector is considered CCP. The employee measures critical limits to assess whether a CCP is under control. If the system is not achievable, appropriate corrective actions are immediately taken.

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86

Processing Formulated Fish and Fish Products

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86.1 Introduction 916
86.2 Quality Parameters of the Formulated Products 916
86.2.1 The Ingredients 917
86.2.1.1 Fish and Mince Meat Quality 917
86.2.1.2 Hydrocolloids and Nonfish Proteins 918
86.2.1.3 Chloride Salts 920
86.2.1.4 Batters and Breading Materials 920
86.2.2 Quality Control During the Processing 920
86.2.3 Freezing Process 924
86.2.4 Frozen Storage and Retailing 924
86.2.4.1 Changes in Texture and Physical Appearance 924
86.2.4.2 Lipid Oxidation 925
86.3 Shelf-Life 926
86.4 Product Thawing During Retailing and Home Preparation 926
86.5 Summary and Future Trends 927
References 927

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86.1 INTRODUCTION

Formulated fish and fish products are available in the frozen and dried forms. Two classes of these products exist; one that is formulated with high percentage of protein and the other is formulated with relatively high percentage of starch. Manufacturing of the formulated fish products has become increasingly important due to the varieties it can offer and also due to the abundant of alternative resources. It has been reported that in UK and European waters alone, approximately 100 species are harvested for food (Bayliss 1996). Morales and others (2001) had also reported the abundance of low value warm-water fish species such as Atlantic croaker (*Micropogon undululatus*), Mexican flounder (*Cyclopsetta chittendeni*), and Northern kingfish (*Menticirrhus saxatilis*) having the potential for surimi production.

Frozen entries can be nonbreaded or breaded. The breading materials differ in color, texture, and physical appearance. They can have a whitish to strong orange hue with a breadcrumb or flaky appearance. "Tempura type" coatings are cream in color and flaky. Examples of these products are the fish balls, fish cakes, fish rolls, fish filaments, fish fingers, fish sausages, and nuggets. These products can also be of squid, shrimp and crab flavors. These products are popular in the Asian countries and are gaining popularity in the West, which is perhaps due to their nutritional contents and increasing exposure to the products. They can be deep-fried, boiled, or steamed prior to their consumption. These formulated products are consumed as a menu or part of a dish during dinner and supper or as snacks during tea times. In some regions of the Asian countries, they are also prepared at breakfast. The value adding of the lower quality fish could be the factor that contributed to their growth and development in the Asian countries. The formulated products come in different shapes, sizes and flavors, which depend on the preference of the region. A fish finger in Japan is considered sweet to the South East Asian taste buds. Sugar content in kamaboko (Japanese fish cake) can be as high as 3% and in between 1–1.5% for fish cakes in the South East Asian countries. Aggressive product development has been carried out for these frozen entries. Fish balls formulated with mixed vegetables, corn, and spices are available in the market.

Fish and shrimp crackers are examples of the dried formulated product. They are also distributed in fried ready-to-eat form. "Keropok lekor," a product resembling sausage in texture, is also a formulated product, but it is cannot be categorized as dried product. It can be deep-fried or eaten as it is. Keropok lekor may be known only to the Asean countries. Both fish crackers and keropok lekor are high in starch (as high as 60%). The starch allows the fish crackers to expand during the deep-frying process and gives the crispy characteristics of the products.

86.2 QUALITY PARAMETERS OF THE FORMULATED PRODUCTS

Good Manufacturing Practices and Good Hygienic Practices are always insisted in the manufacturing of all these products. These products when marketed often have to comply with strict regulations impost by local authorities or importing bodies. However, special specifications have to be identified for individual product for in-house quality control. For frozen products, the good freezing practices must be adhered to during the freezing process for the assurance of a high-quality frozen product. Dried products are very sensitive to fluctuations in relative humidity and exposure to light.

Therefore, role of packaging becomes very significant. Storage temperature and duration are other parameters that must be control for the best shelf-life.

Factors that can affect the quality of the products during processing are the (1) ingredients, (2) quality control during the processing proper, (3) type of packaging, and (4) storage. The quality of the product can deteriorate during the distribution and retailing. Abuse in the temperature during transport and retailing are detrimental to product quality, especially the frozen products. Dried or prefried products are more prone to rancidity development.

86.2.1 The Ingredients

86.2.1.1 Fish and Mince Meat Quality. Whole fish is the raw ingredient for the manufacturing of formulated products. Good quality raw material is the first important key in the processing of these products. Raw material must always be fresh and handled at chilled temperature or iced, at the minimum. Studies have shown that poor quality fish are associated with the disintergration of the tissues, the development of spoilage odor, the accumulation of protein breakdown constituents such as the bioactive amines, and discoloration. The rate of deterioration is governed by species and the origin of catch as demonstrated by Johnston and others (1973) in their comparative study of 19 species of fish, whereby the myosins of warm-water species are found to be more stable at temperatures of 0–18°C as compared to fish from temperate waters. Freshness of the raw materials affects their organoleptic properties such as their taste-active compounds (Konosu and Yamaguchi 1990). The rate of freshness loss was reported to be generally slower for warm water fish in 82 species of fish comprising of species from tropical, subtropical, and temperate waters (Tsuchimoto and others 1986). Estimation of fish freshness based on physical attributes such as the eyes, gills, and the skin texture and luster can be carried out by experienced worker or through elaborate procedures such as the K-value determination. A group of researchers have reported the potential of a multivariate approach for freshness evaluation through the combination of the electric nose and the dielectric TDR method (Kent and others 2004). The concentration of biogenic amines has become a major concern of late due to their reported toxicity. Histamine, putrescine, and cadaverine have been suggested as spoilage indicators in fresh food (Saccani and others 2005). In 10 biogenic amines studied in tuna, it was found that the storage temperature had a crucial effect on the amount and the intensity of amine production (Veciana-Nogues and others 2004). Textural characteristics of the final product in products like fish nuggets are dependent on the nature and state of the initial fish tissue. Once general spoilage such as autolysis and microbial spoilage sets in, the quality of the raw material will decrease and this has a negative effect on the final product. No additive or preprocessing treatments can rectify this defect.

Prerigor muscle is always the best starting material for further processing since this is the stage where inosine monophosphate (IMP) is at its highest concentration. The concentration of IMP has been related to the natural sweetness of fish flesh. During rigor, the muscle goes through the natural contraction process, which resembles a tensed up muscle and has its water holding capacity at the lowest. These structural changes have been well demonstrated through electron microscopy in Blue grenadier (*Macruronus novaezelandiae*) (Bremner and Hallett 1985) and in bighead carp (Jamilah 2004). Structural changes of the fish flesh and related biochemical changes such as proteases activities

after postmortem had been discussed by Bremner (1992). This will affect the gelling ability in the fish protein and will be reflected in the lower springiness of the product.

Formulated products are preferred for their unique taste as well as their textural identity. Kyaw (2004) had identified that textural characteristic is the most important criteria in the marketability of these products and the taste factor becomes the second element. The thermal gel forming ability of the myosin in the presence of salt to dissolve the myosin filaments into myosin monomers dictates their textural properties (Tomoko and others 2006).

The raw material for the formulated products can be the mince fish meat or surimi. Minced meat obtained after deboning are not stable as compared to intact fish muscle due to the disruption of the tissue and the exposure of the meat to air (Babbitt 1986). Microbial and chemical stability of mince fish meat deteriorates at a faster rate than a piece of fish meat due to the increase in surface-volume ratio. Discoloration and odor development have been observed in these mince meats which can be due to the oxidation of the blood pigments, if improper handling such as temperature abuse and delay in processing occurred. Complete use of minced meat usage is more common in small-scale production as compared to surimi, which is used in large volume and continuous production line. In some processing line, a precalculated ratio of the minced fish meat is mixed into the formulation to impart taste and color characteristics of the fish species to the product. However, the fish cracker industries prefer the use of mince fish meat since using surimi will incur the need to add flavorings and colorings to overcome the bland characteristics of surimi. The use of minced fish meat renders natural variations in flavor and texture of the finish product. Better control in the overall quality and product uniformity can be expected when surimi is used instead of the minced fish meat, although with the constraints mentioned above. Frozen surimi blocks of 20 kg are common raw material traded and accounts for approximately 95% of all the surimi produced today. These frozen blocks come in varying grades. Traditionally, surimi is processed from Alaskan Pollock (*Theragra charcogramma*), although in South East Asia, threadfin bream (*Nemipterus japonicus*) surimi is commercially produced. Other species that are utilized for the surimi production in the ASEAN countries are bigeye snapper (*Priacanthus* sp.), jewfish (*Sciaena* sp.), and lizardfish (*Synodu*s sp.) (SEAFDEC 1996). Attempts to produce surimi from other fish species such as Pacific whiting (*Merluccius productus*), menhaden (*Brevoortia tyrannus*), sardines (*Sardinops* sp.), capelin (*Mallotus villosus*), and herring (*Clupea harengus*) have been reported. We have also found that red tilapia (*Tilapia nilotica*) make excellent surimi with hardly any trace of its characterisitic freshwater odor and flavor. In our experience, the use of fatty freshwater fish such as *Pangasius* sp. and the *Clarius batricus* will produce product with poor color and texture.

86.2.1.2 Hydrocolloids and Nonfish Proteins. Hydrocolloids are the next important ingredients that contribute to the texture characteristics and the juiciness of the formulated products. They are added to modify texture and the juiciness of the products. Solid fat are incorporated into burger and sausage formulations to influence the juiciness of finished product. In formulated fish products with relatively high starch contents, these hydrocolloids could act as antistaling agents. Nonetheless, different hydrocolloids will influence these properties to a different extend due to their own inherent properties. Cornstarch, modified starches, tapioca starch, and sago starch are added to varying degree in the formulations since these hydrocolloids have different cold water binding capacities. This

is reflected indirectly in their protein contents, hardness and the springiness of the products. In a market survey of 13 brands available in the Malaysian market of the central zone, the protein content of fish balls ranged from 14.7% to 6.8% and the hardness of the respective products had positive correlations to the protein content (Ismail 2004). Guar gum and *i*-carrageenan are also texture modifiers used in the fish sausage processing industry. They have been demonstrated to attribute to the protection of globular proteins against aggregation during heating through blockage of their hydrophobic binding sites by the bulky polysaccharide moiety thus offering stability to the product (Ibanoglu 2005). In formulated products like keropok lekor where the fish : starch ratio varies from 1 : 1 to 1 : 2, the texture is dependent on the heat-induced gel structure formed by the starch-fish protein interaction. In keropok lekor the addition of locust bean gum, carrageenan, and xanthan gum increased the gelatinization temperatures of sago starch by 4–6°C and the hardness of the gel and the vicsoelastic properties of keropok lekor dough were significantly dependent on the water binding ability of the gel as seen in the change of the loss (G″) and the storage (G′) moduli of the dough (Kyaw 2004). The addition of these hydrocolloids also affects the proneness of the deep-fried products to experience structural collapse after deep-frying and this is evident in their microstructures (Kyaw 2004). Figure 86.1 shows the SEM micrographs of keropok lekor dough formulated with the addition of 3% selected hydrocolloids to highlight the differences in the characteristics of the matrix formed and the different in the size of the cavities which leads to the different structural stability (Kyaw and others 2005). The incorporation of hydrocolloids such as guar and locus bean gums was reported to improve the stability of tapioca starch in products like bread (Guarda and others 2004; Temsiripong and others 2005).

All forms of soy proteins are also added in fish burgers, sausages, rolls and nuggets. In burgers, textured vegetable proteins are preferred. The amount incorporated into these products varies since they have different hardness characteristics. The percentages

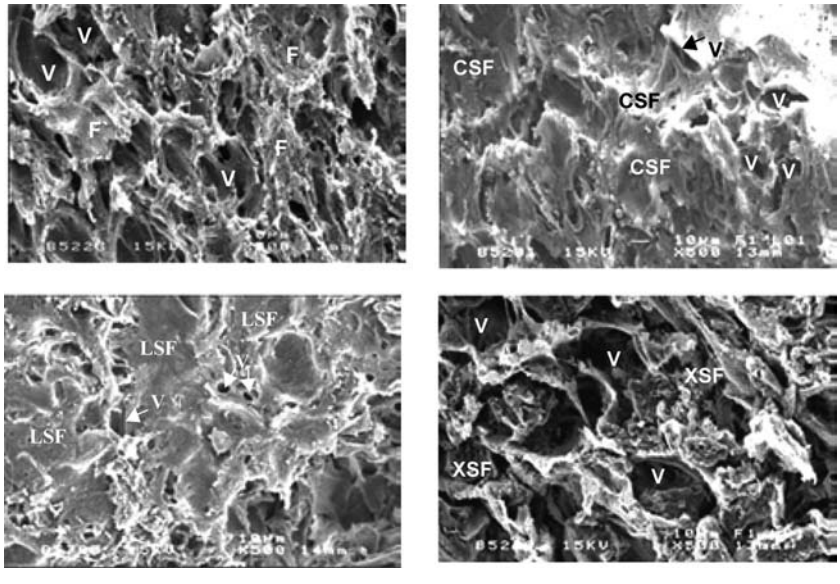


Figure 86.1 *Texture and microstructural characteristics of keropok lekor as affected by 3% incorporation of Food Grade Biopolymers (clockwise: control, carraneenan, xanthan, locust bean gum).*

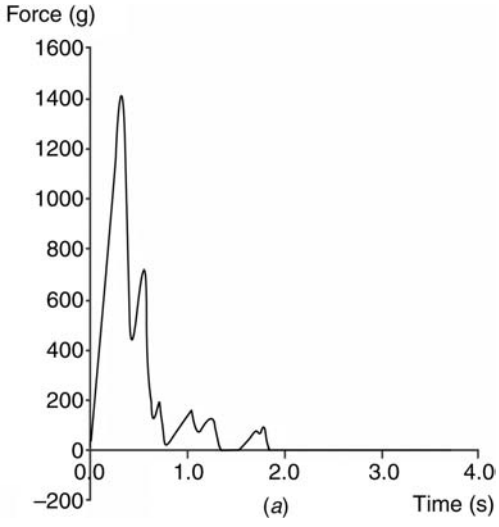
incorporated in these products are lower than those reported for beef-based products. Fish meat has a lot milder taste and aroma and easily override by the beany flavor of the soy protein. Excess amount of soy protein could result in hard textured products and not within the acceptable range of fish-based products. In our laboratory, we have found that soy protein can be incorporated from 3% to 10% by wet weight of the formulation. Lower range is more applicable to fish sausage since this product has a softer texture when compared to the springy texture of fish balls.

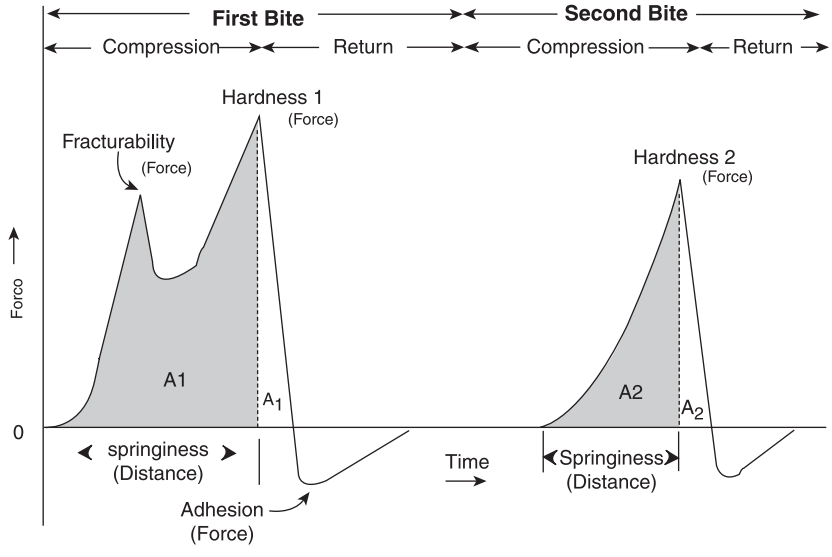
86.2.1.3 Chloride Salts. Sufficient amount of salt is required to help in the solubilization of the fish protein to form the gel. Salt content in the formulated products of high protein content are usually in the vicinity of 2%; beyond which the product may be salty. Optimum concentration of salt in keropok lekor was found to be at 2.5% (Kyaw and others 2003). Using response surface methodology (RSM), the influence of salt and sugar on the textural characteristics of keropok lekor has been demonstrated by Kyaw and others (2003). The salt and sugar concentrations significantly affect the penetration force and springiness in the first order relationship. For the formation of good surimi gels, the addition of 0.2% calcium chloride to Atlantic croaker, Mexican flounder, and Northern kingfish has been recommended (Morales and others 2001).

86.2.1.4 Batters and Breading Materials. Texture of these formulated products is also affected by the freezing techniques and the rates of freezing. In battered products, the batter and the breading material play crucial role in the overall texture perception of the product. Ingredients for batter and breading materials differ from one manufacturer to another. Crude fat and protein content of four local breading materials showed that the fat and protein content varied from as low as 1.14% to as high as 27.7% for fat and 4.1 to 7.7% for protein (Tay 2005). The amount of batter pick up will affect the total cooking loss and the fat uptake by the product. The coating characteristics will also influence the fat uptake during frying.

86.2.2 Quality Control During the Processing

The quality control during the processing can be looked at as four separate components, that is, the formulation, the processing stage proper, the freezing process or the drying stage and the storage and retailing. Quality control (QC) of each product line has to be identified. In any production line the QC is developed to ensure that the specifications and the standards of the products are maintained within a prescribed tolerance limits during all stages from raw material receiving until the product leave the production line. In one frozen reformed seafood processing company, the QC program include checking process at in-coming, in-process, and preshipment, laboratory and sensory test (personal communication). The in-process checking (color, texture, physical attributes such as uniformity of size), chemical analysis and microbial count can be carried out at preidentified intervals. Texture analysis and color monitoring are routinely carried out in frozen and dried products. An example of an instrumental texture profile analysis using Texture Analyser TA-XT 2i (Stable Micro System 1999) for tilapia fish chips (Ho 2003) can be seen in Figure 86.2(*a*) the hardness and the general texture profile for fish chips and other fish products and (*b*) is the sketch for springiness and gumminess calculation for products like keropok lekor. From the deformation curve, the highest peak





Cohesiveness[1,2] = A2/A1 (Ratio)

Gumminess = Hardness x Cohesiveness (Force)
Chewiness = Gumminess x Springiness (Energy)

Notes: 1 Areas as defined by Bourne = A1 and A2
2 Areas as defined by Friedman = (A1 + A_1) and (A2 + A_2)

(*b*)

Figure 86.2 *(a) The texture profile of tilapia chip after frying (profiles were obtained using Texture Analyzer Stable Micro Systems, TA-XT2, England). (b) Force-time curve of texture profile analysis instrument.*

recorded was the force required for breaking the fish chip and it represented hardness. The number of major peaks was considered as an indication of crispiness.

In products like the fish crackers, the crispiness of the product and its linear expansion after frying are important and monitored. Figures 86.3, 86.4(*a*) and (*b*) give an example of a flowchart for the processing of fish balls, fish crackers, and keropok lekor, respectively.

The quantity and the type of binders and texture modifiers incorporated in the formulations are important. Texture of these formulated products is also affected by the freezing techniques and the rates of freezing.

The mixing time, the setting time and temperature (for fish balls), the cooking time or the time required for gelatinization of protein, starch, and the sufficient development of the protein–starch linkages to obtain a desirable texture, the drying time and the thickness (for fish crackers) which will affects its linear expansion and crispiness are among the control points during the processing stage. Variations in the mixing time of these products are mainly due to the size of the processing batch. The setting time and temperature for fish ball type products are dependent on the species. This is because each fish species has an endogenous transglutaminase, which is a calcium dependent enzyme that catalyzes an acyl transfer reaction between gamma-carboxyamide groups of glutamyl residues in proteins and primary amines that is responsible for the setting characteristics of surimi (An and others 1996). In our laboratory, the setting time is 20–25 min and the temperatures are set at 45°C and 55°C for the first and second setting steps when working with surimi from seabream. However, the recommended setting time for some selected fish species surimi from Gulf of Mexico is at 40°C for 30 min and followed by another setting at 90°C for 15 min in the presence of 0.2% calcium chloride (Morales and others 2001). A bigger variation in the cooking time is sometimes obtained which is

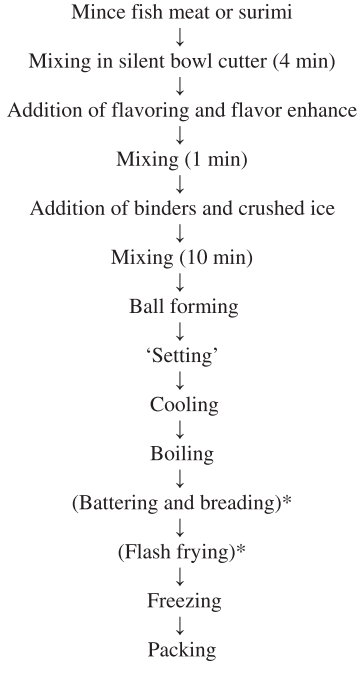


Figure 86.3 *The flowchart for the processing of fish balls. *Only for breaded fish balls.*

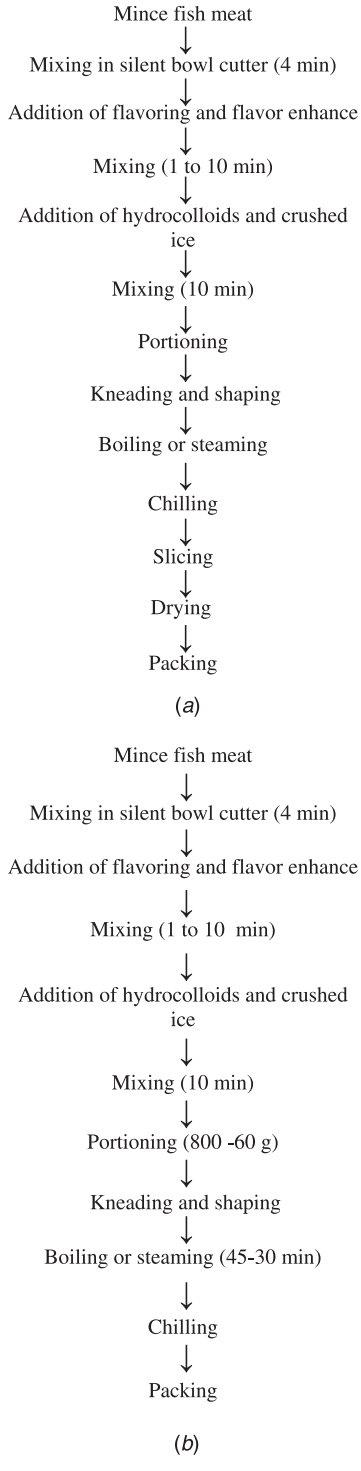


Figure 86.4 *(a) The flowchart for the processing of fish crackers (b) The flowchart for the processing of "keropok lekor."*

due to the variation in the actual ingredients and the physical dimension of the products. The later is more obvious in formulated products with higher starch contents, which may be due to the need for the starch granules to expand prior to their gelatinization.

86.2.3 Freezing Process

The freezing process for formulated products is similar to other food commodities. Ramaswamy and Tung (1984) had documented the review on the mathematical approach of predicting freezing rates of food. Differences may exist in the actual freezing point and the super-chilling phenomena. Freezing rate, which is influenced by the temperature difference between the product and the cooling medium; the modes of heat transfer to, from, and within the product; the size, shape, and type of package containing the product; and the size, shape, and thermal properties of the product (Fennema and Powrie 1964) is important to the products. The knowledge in the heat transfer parameters in terms of thermal diffusity and heat transfer coefficient as well as the estimation of temperature profiles are essential to attain an optimum cooling operation (Abbas and others 2006). A study on the freezing rate of tilapia (*Oreochromis* sp.) muscle by airblast and liquid nitrogen freezing has found that the freezing rate of the muscle is strongly correlated to the freezing temperature, that is, the lower the temperature, the faster is the freezing rate; regardless of the technique used (Chen and Pan 1995). In the manufacturing of fish nuggets, freezing process can also be done prior to packaging and this will reduce the freezing time.

Fast freezing has always been advocated for superior quality product, although freeze-cracking, a condition associated with crust formation, may happen in some cases, for example, freezing whole fish cryogenically has been reported to induce thermal stress and cause a radial cracking and a shattered structure upon thawing (Laverty 1991). However, several reports had implicated that fast freezing will ensure a better storage stability of the fish during frozen storage (Lee 1982; Semenov and others 1986; Yay and Bonie 1997). To date, no report on thermal stress for formulated products is found. Although very rapid freezing techniques can be employed to freeze these products, due to the economics of the production, they are often blast frozen with the exception of the seafood analogs, such as imitation crabmeat and abalone, which are individually quick frozen (IQF).

Rate of freezing determines the rate and size of ice crystals formation. Rapid freezing has always been associated with small ice crystals and thus causing minimal textural damage upon product thawing. Ice crystal formation and moisture migration during freezing was further minimized by the addition of locust bean gum and carboxymethyl cellulose (CMC) for these products with high starch content (Yap 2005).

86.2.4 Frozen Storage and Retailing

86.2.4.1 Changes in Texture and Physical Appearance. During storage and retailing, factors of concern are the general appearance of the product such as the presence of excessive ice crystals in the package and on the product surface, surface discoloration, and integrity of the package. Ice formation in the package and on the product surface is due to moisture migration from inside the product to the outside environment due to the temperature and the vapor pressure difference in the package (Fennema and others 1973; Reid 1997). Quality attributes such as textural

changes, change of flavor and odor are only recognizable upon consumption and prolong storage. An example of textural changes due to excessive storage period was reported for hake (*Merluccius merluccius*) that had been frozen for 2 years at −12°C (Montero and Borderias 1992). Textural changes during frozen storage are in part due to the unfolding of the myosin (Wagner and Anon 1985). The other factor that could cause the textural changes is due to the aggregation of the collagen and the formaldehyde (Montero and Borderias 1989, 1990). The electrophoretic pattern of myosin denaturation of white and red muscles from cod (*Gadus morhua*), tusk (*Brosme brosme*), and capelin (*Mallotus villosus*) also pointed to the different sensitivity of the myosin molecule of different fish species and tissue (Martinez 1992). Product rancidity is only obvious upon prolong storage which may be accompanied by textural changes (Jamilah and Wong 1996). It is also important to have a careful monitoring of warehouse temperature, duration of storage, and logistic. Improper retail handling and display can cause quality deterioration. It has been observed that temperature fluctuation of the frozen product happens quite often during the defrosting of the display refrigerators. The extent of the quality deterioration depends on the number of the freeze–thaw cycles experienced by the product. Freeze stability of these products is also dependent on the water binding agents added such as the addition of natural biopolymers and cryopectants that were incorporated in the surimi (Herrera and others 2000). Loosening of breading material or distorted product shape in formulated products has been observed in the local hypermarkets. Battered products that are frozen after prefrying or cooking require strong coatings to resist subsequent handling steps (Suderman 1993). Prolonged frozen storage for 52–54 weeks at −20°C are more detrimental to fish species from cold water as compared to those from warm water environment based on the enthalpies of the transitions of their myosin in a differential scanning calorimetry (DSC) study (Davies and others 1994). This is perhaps also true for the products derived from them.

86.2.4.2 Lipid Oxidation. Lipid oxidation can cause the development of off-flavor and off-odor or textural changes or the combination of all the changes to a varying degree. During frozen storage, lipid oxidation occurs at slower rate. For products that are battered and prefried or unbreaded but prefried, the lipid oxidation may be relatively more rapids than others. Generic mechanisms for off-flavor development in frozen fish have been proposed, however, the process is too complex; therefore, no generalization should be made (Hedges and Nielsen 2000). Khayat and Schwall (1983) suggested that the lipid oxidation in seafood can be catalyzed by metal ions and the oxidized lipids bind with protein to form lipid–protein complexes that are responsible for the change in quality such as toughening, development of unacceptable flavor and odor. Lipid oxidation and enzymatic peroxidation have been demonstrated to be temperature dependent as seen in the work on whole and minced rayfish (*Raja clavata*) muscle where more significant changes at −18°C was obtained as compared to that at −40°C for their respective lipid compositions (Fernandez-Reiriz and others 1995) and in catfish (*Ictalurus puntatus*) was enzymatic activities were reported to be inhibited at frozen temperatures below −10°C (Eun and others 1994; Haard 1994). Cold-store flavor developed in frozen cod (*Gadus morhua*) was attributed to the lipolysis in the phospholipid fraction to produce hept-*cis*-4-enal (Hardy and others 1979). However, extensive lipid oxidations in formulated products are not reported since their market turnover is normally within a few months.

86.3 SHELF-LIFE

Textural changes are often the main limiting factor for frozen formulated products; however, in products with high percentage of starch, the development of rancidity and staling are often the deciding factors. Shelf-life of the formulated products is the cumulative effect of their ingredients, preservatives added, the processing protocol, and the quality control of postprocessing handling. For example, Jamilah (1983) had reported a reduction in the microbial load and chemical changes when steaming was adopted instead of boiling for the processing of keropok lekor. Whey protein concentrate and soy protein isolate were reported to improve textural property and suppressed lipid oxidation in cooked meatballs (Ulu 2004). However, no similar study has been reported for formulated fish products. Conventionally, frozen surimi is stabilized by the addition of cryopectants such as sorbitol, sucrose, and polyphosphates; however, research on lizard fish (*Saurida wanieso*) surimi had proved that fish protein hydrolysate exhibits similar cryoprotective effects (Khan and others 2003). Therefore, the use of surimi as the base ingredient instead of mince fish meat indirectly gives an added advantage to the product. Studies on the mechanism of quality deterioration and factors affecting shelf life in frozen storage been published and it is reckoned that sensory studies using panelists are required to supplement this information (Jul 1984). Jamilah (2004) had summarized the shelf-life of the formulated products. The approximate shelf-life of these products is in the vicinity of 6–12 months at storage temperatures of $20 \pm 2^{\circ}$C. For frozen entries, the shelf-life of these products can be extended by the proper choice of packaging for physical protection as well for reduction of negative chemical reactions. In some retail packages of 250 g, a rigid thermoforming plastic tray is used to support the product. Vacuum packaging has been used for these products, but they are not popular; possible product deformation may occur. Theoretically, metal laminates are excellent barrier for gaseous and moisture transfer, yet it is not commonly used.

Concern of safety issues in all seafood and their products are of public interest. Ready-to-eat fish or fishery products, scombrotoxin-forming species, stuffed seafood products, vacuum or modified atmosphere package fish, and raw fresh or frozen shellfish are listed in the "substantial risk potential" seafood (Kvenberg and others 2000). However, freezing the seafood make it less vulnerable as a source of food pathogen if strict quality control is adhered to and the cold-chain is not been broken. Negligible microbial damage to the frozen food during frozen storage can happen since at these temperatures growth rates are very slow and generation times may be more than 100 h (Brown 1991).

86.4 PRODUCT THAWING DURING RETAILING AND HOME PREPARATION

Product thawing is not recommended for the formulated frozen products prior to preparation. This is because during thawing, the product temperature experiences a gradual drop; hence, it passes through a temperature range similar to those temperatures of chilled storage, which can harbor a host of pathogenic bacteria (Brown 1991). For example, *Listeria monocytogenes* showed ability to initiate growth in thawing condition even though the cells were injured during frozen storage (Golden and others 1988). The present principle of handling prior to the final preparation that is "from freezer to cooking." During retailing, unwanted thawing should be avoided

in all occasions. Loosening of the breading material breaded products could take place in excessive thawing.

86.5 SUMMARY AND FUTURE TRENDS

Formulated fish and fish products are definitely going to be one of the products that are processed globally in the future. This is based on the trends that are observed in consumer consciousness in eating healthier foods, omega-3 intake and the globalization of food consumption due to increase exposure to these products. An analogy of this situation is the burger (beef), fries, sausages, and potato chips industry, whereby, it is no longer limited to the Western hemisphere. However, the development of the local industries may evolve into processing formulated products that are adopted and adapted to the local preference for flavor and preparation style. The growth in this industry is also perpetuated by need to increase utilization of underutilized and low value fish.

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87

Flavorants from Seafood Byproducts

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87.1 Introduction 932
87.2 Seafood Byproducts 932
87.3 Flavorings Produced by Enzymatic Hydrolysis 933
87.3.1 Amino Acids 935
87.3.2 Peptides 936
87.3.3 Volatiles 937
87.3.3.1 Potato-Like Odor 938
87.3.3.2 Popcorn-Like Odor 938
87.3.3.3 Cucumber and Green-Like Odors 940
87.3.3.4 Malty, Caramel, and Sweet-Like Odors 940
87.3.3.5 Mushroom-Like Odor 941
87.3.3.6 Other Odors Contributing to Seafood 941
87.4 Summary 942
References 942

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

87.1 INTRODUCTION

The flavor of seafood is influenced by a variety of volatile and nonvolatile flavor components formed via complex reactions caused by enzymatic reactions, lipid autoxidation, microbial action, and process-related heat induced reactions (Lindsay 1990; Kawai 1996; Olafsdottir and Fleurence 1998). Proteins and lipids are the main precursors for the flavor components produced during storage, processing, or cooking. Nonvolatile compounds such as free amino acids, peptides, organic bases and acids, nucleotides, sugars, and inorganic salts influence the taste whereas volatile compounds are responsible for the aroma. The degradation of polyunsaturated fatty acid by lipoxygenase is very important for the production of fresh seafood flavor (Josephson and others 1984a; Josephson and Lindsay 1986; Hsieh and Kinsella 1989), while Maillard and Strecker reactions play important role in developing the aroma of processed or cooked seafood (Baek and Cadwallader 1997). Desirable and most characteristic aromas of crustaceans are generated by thermal reactions during cooking (Spurvey and others 1998).

Flavorants with characteristic seafood flavor can be produced by enzymatic hydrolysis from for example seafood byproducts (In 1990; Pan 1990; Baek and Cadwallader 1999). Amino acids and peptides are released during hydrolysis and influence the taste of the flavorants, while volatile degradation compounds generated in the process give the aroma. An overview of the production of seafood flavorants and the development of key flavor components will be given herein. Examples of the production and characteristics of seafood flavorants will be given from a study done in collaboration with an Icelandic marine biotechnology company Primex ehf (www.primex.is). The aim of the study was to characterize the flavor compounds in seafood flavor powder products produced from, for example, fish cut-off, fish skin and bones, shrimp shell, scallop, and whey using different kinds of endopeptidases and exopeptidases.

87.2 SEAFOOD BYPRODUCTS

Utilization of fishery byproducts and under-utilized species is an important subject because of limited fish resources. Fish industry is economically very important in Iceland accounting for 62% of the value of exported products. The fisheries are divided into demersal fisheries, pelagic fisheries, and shellfish fisheries and the total catch was 2 million tons in 2001. The main commercial fish species belong to gadoids, that is, cod, haddock, pollock, and blue whiting, providing about 66% of the total catch value. Other important species are capelin, herring, ocean perch, Greenland halibut, and shellfish like shrimp, lobster, and scallop. Approximately 25–30% of total catch can be considered as underutilized (Venugopal and Shahidi 1995; Arason 2003). Fisheries byproducts and pelagic fish are a valuable source of digestible proteins of high nutritional quality but they are mainly used for low priced products like fish meal and oils. The fish byproducts are sources of components like lipids, proteins, flavorants, minerals, carotenoids, enzymes, and chitin, which can be used for different applications, including functional foods, healthcare products, pharmaceuticals, and cosmetics. A small percentage is processed into higher valued items, such as seafood flavorants, colorants, chitosan, and enzymes (Shahidi 2003). Enzymatic hydrolysis to produce fish protein hydrolysates and seafood flavorings from various sources of seafood byproducts is one of the approaches for upgrading byproducts. As an example, fish cut-offs from lean species brought to shore by

Icelandic freezing trawlers amounted to about 2000 tons in 2001 (Arason 2003). This raw material is an excellent source for the production of flavorants because problems of lipid oxidation are reduced in lean fish.

The biggest market for marine extracts and powder are fish soups and sauces, about 75% of the total market. Flavorants can also be used as additives in surimi-based products and cereal-based extrusion products like shrimp chips (Haard 1992). In 2000, the global food flavoring market was worth USD 6.5 billion. Savory flavors, including marine products (8–12%) were worth USD 1.5 billion. The market for marine extracts and powder was around 55,000 and 7000 tons, respectively. Industrial users of processed flavors are international food companies like Nestlé (Maggi), Unilever (including Knorr and Royco), Campbell, Kraft, IFF, and Mars (www.rubin.no; www.ubic-consulting.com).

87.3 FLAVORINGS PRODUCED BY ENZYMATIC HYDROLYSIS

Traditional enzymic processes of marine raw materials include fish fermentation, fish sauce, and fish silage production. In these processes endogenous proteolytic enzymes cause partial hydrolysis of the tissue (Gildberg 1993). The use of added chemicals or enzymes to prepare protein hydrolysates from fish and shellfish has been studied for decades. The functionality and the applications of fish protein hydrolysates (FPH) as ingredients for food formulations to improve nutritional value of protein sources in specialized foods is of increased interest (Skanderby 1994). FPH are proteins broken down into peptides of varying sizes with the aid of chemicals or enzymes (proteases). Kristinsson and Rasco (2000) give an excellent review of the production of FPH, and their biochemical and functional properties. Physicochemical and functional properties like solubility, water-holding capacity, emulsifying and foaming properties, fat absorption, and sensory properties are discussed. The main drawback for the use of FPH in food formulations is a problem with bitter taste.

A similar process as for FPH can be applied for the production of flavorants from seafood byproducts (In 1990). The use of seafood byproducts like fish cut-offs for the production of seafood flavorants powder with acceptable sensory properties and solubility has been developed by Primex. The flavorants are produced by controlled proteolytic hydrolysis coupled with other processing methods using different kind of commercial enzymes. Figure 87.1 shows an overview of the production of seafood flavorants and how proteins and lipids act as precursors for the development of components characterizing the taste and aroma of flavorants. The process consists of liquefaction of the raw material by enzymatic hydrolysis, thermal inactivation of the enzymes followed by centrifugation or filtration of bones and shells, and concentration of the flavorants. The final product consists of spray-dried powder of seafood flavorant. The details of the enzymes used and process parameters are not given herein since these are part of the products' specifications and recipes of the company.

The proteases used for the hydrolysis are characterized by their hydrolyzing mechanism into endopeptidases or exopeptidases. The endopeptidases provide peptides with different chain length while exopeptidases systematically remove amino acids (Shahidi 2003). The amino acids and peptides that are released by the proteases can give pleasant-tasting flavor compounds or act as precursors for desirable flavor. The Maillard reaction, including Strecker degradation, involves the reaction between reducing sugars and amino compounds and through numbers of reactions, flavor compounds are generated (Bailey 1998). Amino

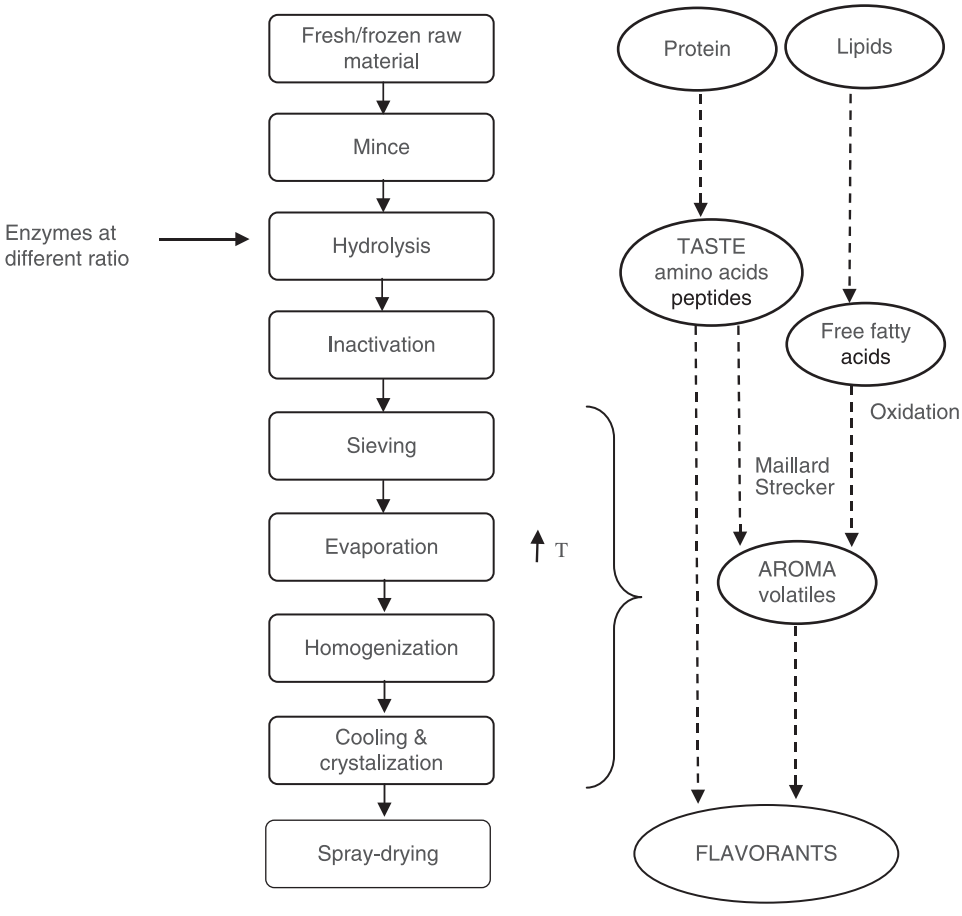

Figure 87.1 *A production scheme for seafood flavorants and the development of taste and aroma active components in the process.*

acids and peptides that are released during enzymatic hydrolysis are therefore important contributors to the flavor and aroma of seafoods. Baek and Cadwallader (1996) found a great amount of thermally generated aroma-active compounds via the Maillard reaction like pyrazines, in enzymatically hydrolyzed crayfish processing byproducts. Numerous volatiles derived from amino acids degradation, contributing to seafood aromas, have been identified in spiny lobster (Cadwallader and others 1995) and in blue crab claw meat (Chung and Cadwallader 1994, 1995).

The sensory quality of flavorants depends on several parameters where the specificity and the level of enzymes are important factors. Other factors such as the pretreatment of the raw material prior to hydrolysis, substrate to water ratio, pH, and temperature also play an important role. Besides the development of desirable flavors produced by enzymatic hydrolysis, formation of taste defects like bitterness caused by bitter amino acids and peptides, burnt taste and ammonia smell, can be a problem (In 1990; Kristinsson and Rasco 2000). Oxidation of fat can also cause off flavors especially in products from fatty species.

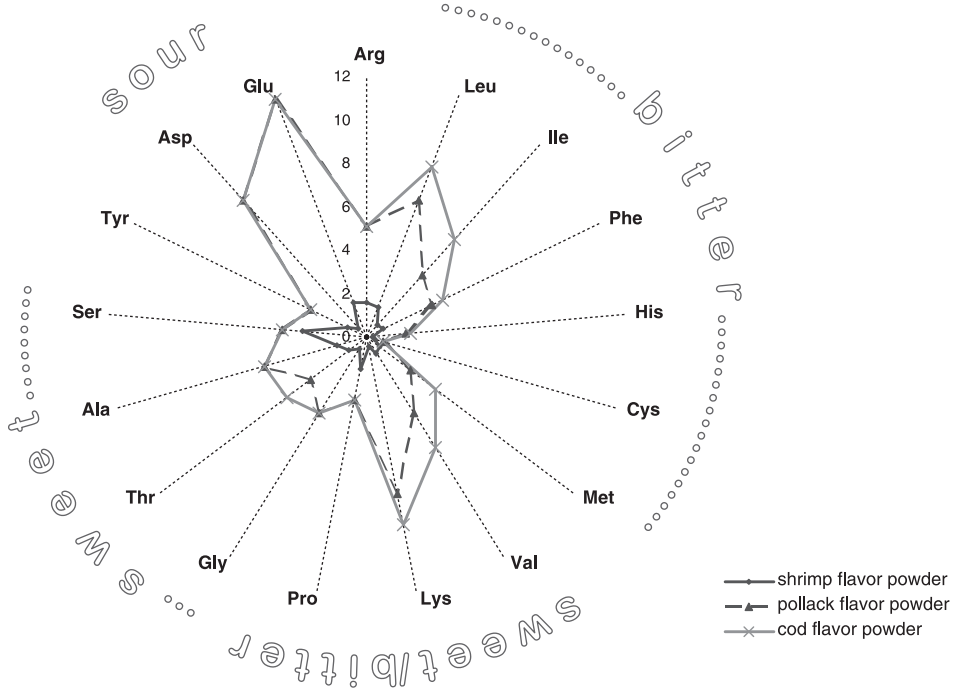

Figure 87.2 *Contribution and composition of free amino acids and their taste in flavorants produced from pollock, cod, and shrimp byproducts.*

87.3.1 Amino Acids

The free amino acids content of flavorants produced by enzymatic hydrolysis can vary greatly, depending on, for example, raw material and enzymes used, thereby influencing the flavor differently. Free amino acids can significantly influence the taste of foods. Individual amino acids have specific taste characteristics and usually they taste either bitter or sweet. Imm and Lee (1999) detected high percentages of free amino acids giving both umami and sweet tastes in hydrolysates from red hake. In the study of seafood flavorants, pollock and cod flavor powder contained about 85% of protein; thereof a very high content of free amino acids (78%) (Jonsdottir and others 2002). Shrimp flavor powder produced from shrimp byproducts contained 71% protein; thereof a much lower content of free amino acids (18%) compared to the cod and pollock flavorants as can be seen in Figure 87.2. The shrimp flavorant was hydrolyzed with endopeptidases but the pollock and the cod flavorants with a mixture of endopeptidases and exopeptidases resulting in a higher concentration of free amino acids.

Figure 87.2 shows that the amino acid composition of cod and pollock flavorants is very similar. The main free amino acids were glutamic and aspartic acid that can give sour taste and their salts give umami taste (Shallenberger 1993). Lysine and valine giving sweet taste with a bitter aftertaste (Haefeli and Glaser 1990) were in high concentrations. The content of leucine and isoleucine were also relatively high. Serine with sweet/bitter/sour characteristics was in the highest amount in the shrimp powder (3%) and proline with a positive sweet/bitter taste (Haefeli and Glaser 1990) was also found in a relatively high concentration. Glutamic acid, arginine, and leucine were detected in relatively high levels but

TABLE 87.1 Taste Assessment of L-Amino Acid Crystals with Respect to the Four Basic Tastes, Hedonic Assessment of Their Taste Attributes and Taste Threshold

Amino Acids	Abbreviation	Taste of L-[a]	Taste Threshold (in mM)
Arginine	Arg	Bitter (−)	2.65
Leucine	Leu	Bitter (−)	10.6
Isoleucine	Ile	Bitter (−)	13.0
Phenylalanine	Phe	Bitter (−)	10.0
Histidine	His	Bitter (−)	21.0
Tryptophan	Trp	Bitter (−)	3.38
Cysteine	Cys	Bitter/sweet (−, S)	1.98
Methionine	Met	Bitter/sweet (−, S)	12.7
Valine	Val	Sweet/bitter (−)	10.6
Lysine	Lys	Sweet/bitter (−)	2.97
Proline	Pro	Sweet/bitter (+)	16.1
Glycine	Gly	Sweet (+)	15.4
Threonine	Thr	Sweet (+)	25.4
Alanine	Ala	Sweet (+)	7.17
Serine	Ser	Sweet/bitter/sour (+)	11.4
Tyrosine	Tyr	Insipid (×)	7.65
Aspartic acid	Asp	Sour (+)	4.0
Glutamic acid	Glu	Sour (+)	4.0
Asparagine	Asn	Bitter/sweet (+)	67.0
Glutamine	Gln	Salty/bitter (−)	43.0

[a] + = pleasant; − = unpleasant; × = unclassifiable; S = sulfurous.
Source: Adapted from Haefeli and Glaser (1990).

other free amino acids were in lower concentrations (<2%). Table 87.1 shows the taste assessment of L-amino acids from Haefeli and Glaser (1990) who studied of the D-, DL-, and L-forms of the 20 primary amino acids. The table summarizes the four basic tastes, hedonic assessment, and the taste-recognition thresholds of the taste attributes of the L-amino acids. The hedonic assessment of the amino acids give information about pleasant (+), and unpleasant (−) flavor perception. Glycine, alanine, and threonine have a sweet taste with pleasant perception but hydrophobic amino acids such as arginine, leucine, phenylalanine, and valine contribute to the bitter taste with unpleasant perception (Haefeli and Glaser 1990; Fuke 1994). The cod and pollock flavorants had mild sweet seafood like sensory characteristics while the shrimp flavorant had more predominant meat like character compromised with typical shellfish flavor. The amino acid composition of the flavorants can partly explain the sensory qualities, for example, high levels of glutamic and aspartic acid with sour taste contribute to a pleasant perception in cod and pollock flavorants. Leucine and isoleucine on the other hand contribute to the bitter taste and unpleasant perception in the flavorants. The complex taste perception of seafood flavorants can also be explained by the presence of peptides contributing to the flavor.

87.3.2 Peptides

Peptides have been recognized for a long time as important contributors to flavor in processed food. Peptides, released by enzymatic hydrolysis or by thermal degradation, can act as flavor precursor in thermally induced reactions (Rizzi 1989). Dipeptides can contribute to the organoleptic characteristics of food where the synergy of different

factors like pH, salt, and concentration of each taste or flavor enhancing component, that is, dipeptides and amino acids, play an important role (Kato and others 1989).

Peptides are polymers of up to 50 amino acids. When the degree of polymerization exceeds 50, the peptide is considered to be a protein (Shallenberger 1993). Kirimura and others (1969) characterized the tastes of 60 different peptides and classified them into three main groups: sour, bitter, and little or no taste. They reported that a relationship existed between amino acid composition and the taste of dipeptides. Dipeptides containing two acidic amino acids, acidic- and neutral amino acids, or acidic- and aromatic-amino acids tasted sour while dipeptides containing neutral amino acids with either large alkyl groups ($\geq$C3) or a combination of large and small alkyl groups ($\leq$C2), neutral and aromatic amino acids, or neutral and basic amino acids tasted bitter. Dipeptides having a little or no taste were composed of two amino acids with small alkyl groups, acidic- and basic amino acids, or two aromatic amino acids. Almost all peptides of hydrophobic L-amino acids evoke a bitter taste and the amino acid sequence of peptides also play an important role in the intensity of the bitter taste (Kato and others 1989; Saha and Hayashi 2001). Ney (1979) reported that the bitterness of peptides depended on their hydrophobic value Q. He suggested to obtain average hydrophobicity Q by summing the hydrophobicities of the amino acid side chains of a peptide and dividing by the number of the amino acid residues. Peptides with Q-values below 1300 are not bitter, whereas peptides with Q-values higher than 1400 are bitter. This principle is valid for molecular weights up to approximately 6000 Dalton, above this limit peptides with Q 1400 are also not bitter.

Uncontrolled or prolonged hydrolysis during the development of FPH and flavorants may cause taste defects like bitterness caused by bitter amino acids and short-chain peptides (Venugopal and Shahidi 1995). Hevia and Olcott (1977) identified basic bitter tripeptides that contributed to off-flavor in fish protein hydrolysates. The tripeptide had aspargine as the middle amino acid and lysine as the C-terminus, with leucine or glycine as the N-terminus. By using exopeptidases N-terminal amino acid residues can be selectively released from polypeptides or proteins resulting in reduced bitterness of protein hydrolysates (Saha and Hayasi 2001; Raksakultahi and Haard 2003). Addition of exopeptidases to modify food is becoming increasingly common, where commercial exopeptidases produced by microorganisms or exopeptidases naturally occurring in foodstuff are applied. Haard (1998) reported that the mid gut gland of marine invertebrates contains a wide range of amino- and carbopeptidases which may find use in debittering of protein hydrolysates.

87.3.3 Volatiles

Numerous volatile compounds have been identified in enzymatically produced seafood flavorants, for example, aldehydes, alcohols, ketones, sulfur containing compounds, and pyrazines (Pan and Kuo 1994; Baek and Cadwallader 1996). During processing a series of changes caused by thermal degradation, lipid oxidation, and Maillard reaction play important roles in generating complicated volatile compounds contributing to the processed flavor. The role of lipid derived unsaturated aldehydes, alcohols, and ketones in seafood flavors are well known. Compounds derived from lipoxygenase initiated oxidation of polyunsaturated fatty acids contribute to the characteristic fresh fish flavor (Josephson and others 1984a), in oxidized fish oils these compounds cause rancid odor (Karahadian and Lindsay 1989) and they also contribute to boiled odor of fish

(Milo and Grosch 1995). Volatile compounds characteristic for processed seafood odors such as alkyl-pyrazines and sulfur-containing compounds have been found in cooked crustaceans, and furans have been found in spray-dried shrimp powder and shrimp hydrolysate (Pan and Kuo 1994). Various methods have been used for isolating and analyzing volatile components from seafood. Gas chromatography is commonly used and the efficiency of the analysis depends on the sampling techniques (i.e., the extraction or concentration methods) and the detection approach. Gas chromatography-olfactometry (GC-O) is very useful for analysis of volatile components with low odor thresholds but gas chromatography-mass spectra (GC-MS) for verification of the components (Jensen and others 1998).

Table 87.2 shows the characteristic odors and volatile compounds identified in the cod, pollock, and shrimp flavorants by GC-O and GC-MS together with odor thresholds and possible precursors. The low flavor threshold of some of the compounds make them potential flavor substances even in trace amount. Aldehydes appear to be the main contributors to the odor but ketones, alcohols, and sulfides were also detected. The most characteristic odors of the seafood flavorants were potato-like odor, popcorn-like odor, cucumber- and green-like odors, caramel- and sweet-like odors, and mushroom-like odor. Some of the compounds responsible for these odors were present in very low concentrations and the quantification was only possible by using GC-O because of their low odor threshold. Figure 87.3 shows the relative odor impact of the key aroma compounds found in cod, pollock and shrimp flavorants.

87.3.3.1 Potato-Like Odor. The most characteristic odor of all the flavorants was identified as potato-like odor (Fig. 87.3). The compounds contributing to this odor were identified by GC-MS as a combination of *cis*-4-heptenal and methional. Methional (3-(methylthio)-propanal), the Strecker aldehyde produced from methionine, has been identified as an important contributor to the aroma of various crustaceans and flavor concentrates (Chung and Cadwallader 1995; Baek and Cadwallader 1997; Kim and others 2000). It has a characteristic potato-like odor and a very low threshold value (Table 87.2). McGill and others (1974) described the odor of *cis*-4-heptenal being similar to that of boiled potatoes. It is a lipid derived compound that can have a cardboard-like odor but in higher concentrations it gives a painty, putty- or linseed oil-like odor (Lindsay 1990). *Cis*-4-heptenal can also contribute to potato-like odor when detected in a low concentration (Josephson and Lindsay 1987). Thus, *cis*-4-heptenal together with methional have been identified as the major contributors to the characteristic potato-like odor of, for example, cooked mussel extracts (Le Guen and others 2001), cooked spiny lobster tail meat (Cadwallader and others 1995), and cooked blue crab claw and lump meats (Chung and Cadwallader 1995).

87.3.3.2 Popcorn-Like Odor. 2-Acetyl-1-pyrroline, giving a popcorn-like odor, was detected as one of the key aroma compounds in the cod, pollock, and shrimp flavorants (Fig. 87.3). Characteristic popcorn-like odor in shrimp was suggested to originate from the shell since 2-acetyl-1-pyrroline was only identified in unpeeled shrimp in a study comparing the volatile profiles of peeled and unpeeled shrimp (Jonsdottir and others 2002). 2-Acetyl-1-pyrroline can be thermally generated and may be formed via the Maillard reaction of glucose and proline (Baek and Cadwallader 1997). 2-Acetyl-1-pyrroline has been identified as an important character-impact aroma compound in enzyme hydrolyzed oyster cooker effluent (Kim and others 2000), cooked spiny lobster tail (Cadwallader and others 1995) cooked blue crab claw and lump meats (Chung and Cadwallader

TABLE 87.2 Volatile Compounds Identified in Cod, Pollock, and Shrimp Flavorants by GC-MS and GC-O Using a Purge and Trap Technique (Tenax)

Compounds	Odors	Precursor[a]	Odor Threshold[b]	Id[c]	RI[d]
cis-4-Heptenal	Potato-like	FA	0.04 ppb (a)	MS, 1, 2	494
Methional	Potato-like	AA, methionine	0.2 ppb (b)	MS, 1, 2	502
2-Acetyl-1-pyrroline	Popcorn-like	AA, proline	0.1 ppb (c)	3	536
2-Acetylthiazol	Nutty-, popcorn-like	?	10 ppb (d)	MS, 1, 2	616
2,6-Nonadienal	Cucumber	PUFA (n-3)	0.001 ppb (e)	MS, 1, 2	760
2-Nonenal	Green, marine algae			MS, 1, 2	769
Hexanal	Green, marine algae	PUFA (n-3)	4.5 ppb (e)	MS, 1, 2	386
2-Methyl propanal		AA, valine		MS	191
3-Methyl butanal	Malty	AA, leucine	0.06 ppm (f)	MS, 1	264
2-Methyl butanal	Malty	AA, isoleucine	0.04 ppm (f)	MS	266
2-Methyl-2-butanal				MS	319
2-Methyl-2-pentenal				MS	403
1-Penten-3-ol	Butter			MS, 1, 2	264
1-Octen-3-ol	Mushroom	PUFA (n-3)	10 ppb (g)	MS, 1, 2	576
Acetone				MS	182
2,3-Butandione	Buttery	FA	2.6 ppb (h)	MS, 1	207
2-Butanone				MS	209
2,3-Pentadione				MS, 1	273
3-Hydroxy-2-butanone	Buttery	FA		MS	282
(1,5-Octadien-3-one)	Geranium	PUFA (n-3)	0.001 ppb (i)	3	581
3,5-Octadien-2-one				MS	681
2-Nonanone	Hot milk			MS	692
2-Decanone				MS	791
2-Undecanone	Orange			MS	884
Dimethyl sulfide	Cabbage			MS, 1	191
Dimethyl disulfide	Putrid, onion-like	AA, methionine	12 ppb (j)	MS, 1, 2	329
Dimethyl trisulfide	Garlic	AA, methionine	0.01 ppb (j)	MS	560
TMA	Fishy, ammonia		30 ppm (k)	MS, 1	173
Heptanal	Fatty			MS	500
Pentanal	Pungent			MS	282
Octanal	Soapy			MS	604
2,4-Heptadienal	Fatty, fishy oxidized flavor			MS, 1, 2	608
Decanal	Soapy			MS	806
Decadienal	Fried fat			MS, 1, 2	906
Benzaldehyde	Almond			MS	557
Benzenacetaldehyde				MS	643
Phenylacetaldehyde	Hawthorn – roses	AA, PHE		3	647

[a]AA: amino acid; FA: fatty acid; PUFA: polyunsaturated fatty acid.
[b]Odor threshold in water: (a) McGill and others 1974; (b) Guadagni and others 1972; (c) Buttery and others 1988; (d) Schutte 1974; (e) Josephson 1991; (f) Sheldon and others 1971; (g) Pyysalo and Suihko 1976; (h) Fors 1983; (i) Swoboda and Peers 1977; (j) Buttery and others 1976; (k) Kawai 1996.
[c]Identified by: MS, mass spectra; 1, standard; 2, GC/O; 3, GC/O and RI references.
[d]Calculated ethyl ester index for DB-5 ms capillary column.

1994), and boiled crab meat of American lobster (Lee and others 2001). 2-Acetylthiazole, also giving a popcorn-like odor, was detected in the flavorant samples derived from shellfish. Many desirable compounds are generated by thermal reactions during cooking of crustaceans, that is, pyrazine and thiazole that give meaty-, nutty-, and popcorn-like odors (Pan and Kuo 1994). Many pyrazine compounds were detected in flavorants produced from crayfish byproducts (Cha and others 1992; Baek and Cadwallader 1996).

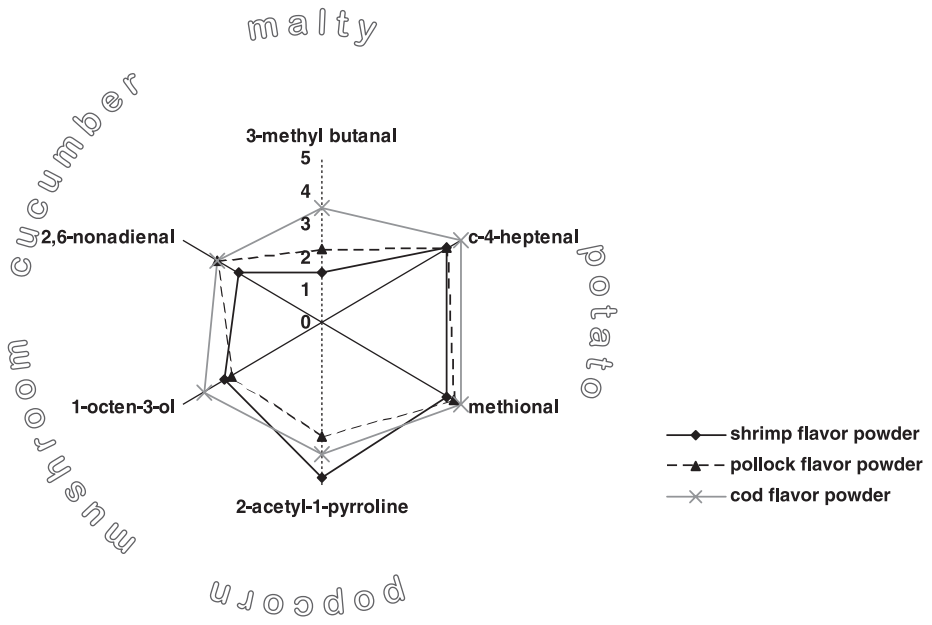

Figure 87.3 *Key aroma compounds and their characteristic odor in flavorants produced from pollock, cod, and shrimp byproducts. Identification by GC-MS and quantification by GC-O (odor scores from 0; not present to 5; strong odor).*

Pyrazine and thiazoles are also important in the development of popcorn-like odor of lobster (Cadwallader and others 1995).

87.3.3.3 Cucumber and Green-Like Odors. Lipid-derived compounds are known to contribute to the aroma of both cooked and fresh crustaceans (Baek and Cadwallader 1997). A desirable sweet, cucumber-like aroma was contributed by *trans*,*cis*-2,6-nonadienal in the flavorants. The compound was present in a low concentration but the aroma impact of the compound was however high because of its low odor threshold (Table 87.2). 2,6-Nonadienal is derived from omega-3 fatty acids via lipoxygenase activity and has been identified in various fish species contributing to green, fresh fish-like, cucumber odors (Josephson and others 1983; Zhang and others 1992; Olafsdottir and others 1997).

Lipid-derived compounds can also cause an undesirable aroma, for example, 2,6-nonadienal can be readily converted to *cis*-4-heptenal through the retro-aldol degradation reaction (Josephson and Lindsay 1987; Cha and others 1997). Other compounds produced because of autoxidation like hexanal, 2,4-heptadienal, and 2,4-decadienal can cause the oxidized, rancid, and painty flavors in fish oils (Karahadian and Lindsay 1989). These compounds were detected in low concentrations in the flavorants and may influence the overall aroma but were not perceived as rancid or undesirable in the flavorants. Other authors have identified similar unsaturated aldehydes in flavor concentrates (Baek and Cadwaller 1996) and hydrolyzed oyster cooker effluent (Kim and others 2000).

87.3.3.4 Malty, Caramel, and Sweet-Like Odors. 2-Methylpropanal, 2-methylbutanal, and 3-methylbutanal are short branched chains that were among the

aldehydes detected in the flavorant samples. These aldehydes give malty-, chocolate-, and sweet-like odor and together with other short chain aldehydes, ketones, and alcohols give a characteristic sweet mild caramel-like odor. Low molecular weight compounds with high volatility are likely to influence the first flavor perception. These compounds can also react further and contribute to the development of other flavor components. 2-Methylpropanal, 2-methylbutanal, and 3-methylbutanal are known as Strecker or microbially derived aldehydes from valine, isoleucine and leucine, respectively. Figure 87.2 shows that the levels of leucine and isoleucine were relatively high in cod and pollock flavorants compared to the shrimp flavorant and as expected their volatile degradation compounds 3- and 2-methyl butanal, respectively, contributing to sweet-, caramel-, malty-like odor were also higher in the cod and pollock flavorants than in the shrimp flavorant (Fig. 87.3). 3-Methyl butanal has been identified as an important contributor to boiled crab meat of American lobster (Lee and others 2001).

87.3.3.5 Mushroom-Like Odor. 1-Octen-3-ol was identified in all the flavorants and contributes to the overall seafood-like odor. Alcohols are generally minor contributors to flavor unless present in relatively high concentrations or if they are unsaturated. The odor threshold for 1-octen-3-ol is 10 ppb (Table 87.2). Is has been detected in low concentrations in seafood flavorants (Baek and Cadwallader 1996) and gives the pleasant mushroom fresh fish odor. Unsaturated alcohols are derived from long chain polyunsaturated fatty acids and contribute to the characteristic fresh mushroom-like odors found in freshwater and seawater fish (Josephson and others 1984b; Lindsay 1990).

87.3.3.6 Other Odors Contributing to Seafood. *Meaty and roasted-like odors*: Pyrazines and sulfur-containing compounds have been shown to play important roles many thermally processed foods. They have been found in both roasted and boiled shrimp and in snow crab cooker effluent and effluent concentrate giving meaty and roasted-like odors (Cha and others 1993; Baek and Cadwallader 1997).

Flowery and fruity-like odors: Many ketones were identified in the flavorant samples, some of them giving a sweet flowery and fruity characteristic often detected in shellfish (Cha and others 1992). Ketones can be oxidation products of polyunsaturated fatty acids or microbial degradation products of amino acids. They have a low odor threshold and can therefore influence the sensory characteristics of food, for example, 1-octen-3-one and 1,5-octadien-3-one, contributing a geranium like odor, have a great impact on the aroma of fresh fish (Josephson and Lindsay 1986). Numbers of ketones have been detected in boiled and roasted shrimp. 2,3-Butandione or diacetyl and 2,3-pentanedione give a buttery-like odor characteristic of shellfish. Diacetyl is generated through the Maillard reaction and is characteristic for cooked seafood (Le Guen and others 2001). Diacetyl was detected as one of the key aroma compounds in cooked spiny lobster tail meat (Cadwallader and others 1995).

Cabbage and onion-like odors: The sulfides detected in the flavorants were dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS). DMDS and DMTS are derived from sulfur-containing amino acids. They all have a very low odor threshold and are therefore likely to influence the overall aroma of food. Sulfides have been detected in most thermally processed crustaceans like shrimps and crabs. Concentrations of DMDS and DMTS in flavor concentrates produced from crayfish-processing byproducts, increased after enzymatic hydrolysis (Baek and Cadwallader 1996).

Fishy-like odor: Production of TMA (trimethylamine) from TMAO (trimethyl amine oxide) via microbial metabolism is well known contributing to fishy aroma and flavors in marine fish and shellfish. The odor of TMA is described as crab-like, old fishy, or fishhouse-like (Lindsay 1990). TMA was detected in some of the flavorant samples. TMA and ammonia develop because of microbial degradation of TMAO and amino acids, respectively, during refrigerated storage of fish and contribute to the stale ammonia like odor of stored seafood products. DMA (dimethyl amine) is produced by endogenous enzymes in the fish and is known to cause flavor problems in frozen fish. These compounds are commonly used as indicators for microbial growth and freshness of fish raw material (Oehlenschläger 1991). The contribution of TMA to the overall odor of the flavorants is believed to be low and Kim and others (2000) showed that the amount of TMA was only a minor part of the extractable nitrogen content in hydrolyzed oyster cooker effluent.

87.4 SUMMARY

The controlled production of seafood flavorants by enzymatic hydrolysis of lean fish cut-offs and byproducts from shrimp processing resulted in cod, pollock, and shrimp flavorants with a characteristic seafood odor and taste. GC-O and GC-MS results indicate that the key aroma compounds identified in the seafood flavorants are produced via Maillard reaction and Strecker degradation of amino acids during enzymatic and thermal processing of the flavors. Lipid derived components also contribute to the aroma of the flavorants. Amino acids and peptides play an important role in taste of the flavorants. To gain more knowledge and understanding of the formation of the flavors the industry can link together information on process parameters, the details of the enzymes used and the composition of key volatiles. It is thus possible to control the process and the development of the key flavor components. The information gained can be used for product formulation and development of recipes. By selection of raw material and variation of process parameters the formation of the desirable aroma of the flavorants can be achieved. The use of byproducts for the production of value-added seafood products like flavorants is a feasible way to reduce the amount of valuable under-utilized raw material.

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88

Seafood Processing: Basic Sanitation Practices

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88.1 Background Information 948
88.2 Fresh and Frozen Fish 950
88.2.1 Sanitation Critical Factors 950
88.2.2 Raw Materials 950
88.2.3 Manufacturing 951
88.2.4 Controls 951
88.2.5 Summary and Checklist 952
88.3 Canned Tuna 953
88.3.1 Sanitation Critical Factors 953
88.3.2 Raw Materials 953
88.3.3 Processing 953
88.3.4 Sanitation 954
88.4 Oysters 954
88.5 Blue Crab (Fresh and Pasteurized) 955
88.5.1 Sanitation Critical Factors 955
88.5.2 Raw Materials 955
88.5.3 Manufacturing Process 955
88.5.3.1 Cooking 955
88.5.3.2 Cooling 956
88.5.3.3 Picking 956
88.5.3.4 Packing 956
88.5.3.5 Pasteurization 956
88.5.3.6 Lighting, Ventilation, Refrigeration, Equipment 957

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

88.5.4 Overall Sanitation 957
88.5.5 Checklist for Crustacea Processor 957
88.6 Scallops 958
88.6.1 Background Information 958
88.6.2 Sanitation Critical Factors 958
88.6.3 Raw Materials 959
88.6.4 Processing 959
88.6.5 Overall Sanitation 959
88.7 Shrimps 960
88.7.1 Sanitation Critical Factors 960
88.7.2 Raw Materials: Receipt and Storage 960
88.7.3 Plant Sanitation 961
88.7.4 Processing 962
88.7.5 Finished Product Process and Quality Assurance 963
88.8 Smoked Fish 964
88.8.1 Sanitation Critical Factors 964
88.8.2 Plant Sanitation and Facilities 964
88.8.3 Raw Materials 965
88.8.4 Processing 965
88.8.4.1 Salting and Brining 965
88.8.4.2 Heating, Cooking, and Smoking Operation 966
88.8.4.3 Cooling 966
88.8.4.4 Packaging 966
88.8.5 Storage and Distribution 966
88.8.6 Laboratory Controls 966
88.8.7 Overall Sanitation 967

88.1 BACKGROUND INFORMATION

The U.S. national regulatory authority for public protection and seafood regulation is vested in the FDA. The FDA operates an oversight compliance program for fishery products under which responsibility for the product's safety, wholesomeness, identity, and economic integrity rests with the processor or importer, who must comply with regulations promulgated by the FDA. In addition, FDA operates the low-acid canned food (LACF) program which is based on the hazard analysis critical control point (HACCP) concept, and is focused on thermally processed, commercially sterile foods, including seafood such as canned tuna and salmon.

The seafood processing regulations, which became effective on December 18 1997, require that a seafood processing plant (domestic and exporting foreign countries) implement a preventive system of food safety controls known as a HACCP plan. HACCP essentially involves: (1) identifying food safety hazards that, in the absence of controls, are reasonably likely to occur in the products; and (2) having controls at "critical control points" in the processing operations to eliminate or minimize the likelihood that the identified hazard will occur. These are the kinds of measures that prudent processors already take. HACCP provides a systematic way of taking those measures that demonstrates to the FDA, customers, and consumers, that the firm is routinely practicing food safety by design. Seafood processors that have fully operating HACCP systems advise us that they benefit in several ways, including having a more safety-oriented workforce, less product waste, and generally, fewer problems.

Most FDA in-plant inspections consider product safety, plant/food hygiene and economic fraud issues, while other inspections address subsets of these compliance concerns. Samples may be taken during FDA inspections in accordance with the agency's annual compliance programs and operational plans or because of concerns raised during individual inspections. The FDA has laboratories around the country to analyze samples taken by its investigators. These analyses are for a vast array of defects including chemical contaminants, decomposition, net weight, radionuclides, various microbial pathogens, food and color additives, drugs, pesticides, filth and marine toxins such as paralytic shellfish poison (PSP), and domoic acid.

In addition, FDA has the authority to detain or temporarily hold food being imported into the United States while it determines if the product is misbranded or adulterated. The FDA receives notice of every seafood entry, and at its option, conducts wharf examinations, collects and analyzes samples, and where appropriate, detains individual shipments or invokes "automatic detention," requiring private or source country analysis of every shipment of product when recurring problems are found, before the product is allowed entry.

Further, FDA has the authority to set tolerances in food for natural and man-made contaminants, except for pesticides, which are set by EPA. The FDA regulates the use of food and color additives in seafood and feed additives and drugs in aquaculture. FDA also has the authority to promulgate regulations for food plant sanitation [i.e., good manufacturing practices (GMP) regulations], standards of identity, and common or usual names for food products.

FDA has the authority to take legal action against adulterated and misbranded seafood and to recommend criminal prosecution or injunction of responsible firms and individuals.

FDA conducts both mandatory surveillance and enforcement inspections of domestic seafood harvesters, growers, wholesalers, warehouses, carriers, and processors. The frequency of inspection is at the agency's discretion, and firms are required to submit to these inspections which are backed by federal statutes containing both criminal and civil penalties.

FDA provides financial support by contract to state regulatory agencies for the inspection of food plants, including seafood.

The FDA also operates two other specific regulatory programs directed at seafood – the Salmon Control Plan and the National Shellfish Sanitation Program (NSSP), recently augmented by the Interstate Shellfish Sanitation Conference (ISSC). These are voluntary programs involving the individual states and the industry.

The Salmon Control Plan is a voluntary, cooperative program among the industry, FDA, and the National Food Processors Association (NFPA). The plan is designed to provide control over processing, plant sanitation, and to address concerns about decomposition in the salmon canning industry.

Consumer concerns about molluscan shellfish are addressed through the NSSP. It is administered by FDA and provides for the sanitary harvest and production of fresh and frozen molluscan shellfish (oysters, clams, and mussels). Participants include the 23 coastal shellfish-producing states and nine foreign countries.

The NSSP was created upon public health principles and controls formulated at the original conference on shellfish sanitation called by the Surgeon General of the U.S. Public Health Service in 1925. These fundamental components have evolved into the National Shellfish Sanitation Program Manual of Operations. A prime control is proper evaluation and control of harvest waters and a system of product identification which enables trace back to harvest waters.

FDA conducts reviews of foreign and domestic molluscan shellfish safety programs. Foreign reviews are conducted under a Memorandum of Understanding (MOU) which FDA negotiates with each foreign government to assure that molluscan shellfish products exported to the United States are acceptable.

FDA's regulations on HACCP for seafood processing have been in full force since 1997. HACCP, in addition to other scientific and technical considerations, is an extension of the basics of food processing sanitation which uses FDA's current good manufacturing practice regulations (CGMPR) and the Food Code as frames of references. The FDA considers such sanitation compliance a prerequisites to HACCP planning and implementation.

This chapter discusses those prerequisites of basic sanitation for seafood processing. If you are a seafood processor and you are planning to start the HACCP program, you must first examine the current practices of your operation to ascertain that it complies with such prerequisites.

The information presented in this chapter has been modified from the CGMPR of the FDA and USDA, the Food Code, and other documents issued by the FDA on inspection of seafood processing plants.

The format and style used in this chapter reflects the instructional process between a teacher (e.g., a training supervisor) and student (e.g., a company personnel).

88.2 FRESH AND FROZEN FISH

88.2.1 Sanitation Critical Factors

The critical factors to remember when a company officer performs a sanitation inspection of a processing plant for fresh and frozen fish.

1. Look for evidence of rodents, insects, birds, or pets within the plant.
2. Observe employee practices including hygenic practices, cleanliness of clothing, and the use of proper strength hand-dip solutions.
3. Check to see if fish are inspected upon receipt and during processing for decomposition, off odor, parasites, and so on.
4. Check for decomposition and parasites during EI.
5. Ascertain if equipment is washed and sanitized during the day and at the beginning and end of the daily production cycle.
6. Check if the fish are washed with a vigorous spray after eviseration and periodically throughout the process prior to packaging.
7. Determine the method and speed of freezing for frozen fish and fish products.
8. Check use of rodenticides and insecticides to assure that no contamination occurs.
9. Observe handling from boats to finished package and observe any significant objectionable conditions.

Specific details on the sanitation are as follows.

88.2.2 Raw Materials

1. Determine what tests are conducted on incoming fish for decomposition, parasites, chemical contamination, and so on.

2. Determine disposition of incoming fish which have been found to be decomposed, contain excessive parasites or contaminated with mercury, pesticides, and so on.
3. Conduct organoleptic examination of incoming fish or fish products, especially those which have been thawed for processing or held for prolonged periods of time at room temperature during processing.
4. Give attention to fish arriving at the plant, as to effectiveness of elimination of decomposed fish, and check fish actually being packed. Determine percentage of decomposed units encountered, classifying each as passable (class 1), decomposed (class 2), or advanced decomposed (class 3).
5. Examine susceptible fish for parasitic infestations (e.g., white fish, rose fish, tullibees, ciscos, inconnus, bluefish, herring, etc.).
6. Check other raw materials and storage areas for rodents, insects, filth, or other contaminating factors.
7. See required specification on other raw materials for bacterial load, and so on (e.g., received under a Salmonella-free certificate issued by a recognized government or private agency).
8. Check for misuse of dangerous chemicals including insecticides and rodenticides.
9. If fish is received directly from boats, see if hook is used for loading and unloading, or for that matter, if a hook is used for any handling of the fish.

88.2.3 Manufacturing

1. Study manufacturing procedure. Include flow plan.
2. Study type of equipment used as to construction, materials, ease of cleaning, and so on.
3. Observe equipment cleaning and sanitizing procedures, and evaluate its adequacy.
4. Observe evisceration procedure, filleting procedure, or other butchering procedures used.
5. Determine source of water used in operation. Check that only potable water from an approved source is used.
6. If, during processing of fish, there are long delays at room temperature, check for decomposition.
7. Examine all handling steps and intermediate steps in processing that could lead to the contamination of the fish with filth and/or bacteria.
8. Study holding times and temperatures during the processing operation.
9. If battering and/or breading fish is involved, check process carefully. In addition, check times and temperature, and for other possible routes of filth and/or microbial contamination.
10. Evaluate compliance with good manufacturing practices.

88.2.4 Controls

1. Check coding system. If no code marks are used, mark suspect lot packages with fluorescent crayon for later sampling.

2. Review records regarding finished product assay for decomposition, parasites, microbial load, pesticides, mercury, and for other quality factors.
3. Study labeling used on products.
4. Check use of preservatives on fish or ice.

88.2.5 Summary and Checklist

Check on

1. Compliance with CGMP.
2. Use of adequate and proper strength hand and equipment sanitizing solutions.
3. Proper cleanup.
4. Evidence of rodents, insects, birds, domestic animals, or any other source of contamination.

Use the following list of indicators of sanitation to make a valid assessment of the operations at different stages of the process flow.

Stage	Assessment
Receive (unload fish)	1. Determine condition of the fish (acceptable or decomposed). 2. Separate work area.
Store	1. Suitable storage area (sanitation). 2. Time/temperature (icing) (quality). 3. Separate work area.
Wash	1. Remove surface slime and dirt (sanitation). 2. Use of potable water.
Filet	1. Personnel sanitation. 2. Equipment sanitation. 3. Separate work area.
Skin (either hand or machine)	1. Personnel sanitation. 2. Equipment sanitation. 3. Separate work area. Same area as fillet operation.
Rinse	1. Potable water. 2. Equipment sanitation. 3. Time/temperature (quality).
Pack (either retail or block)	Equipment sanitation. Personnel sanitation. Suitable packaging materials. Time/temperature (quality). Separate work area.
Freeze	Time/temperature (quality)

88.3 CANNED TUNA

88.3.1 Sanitation Critical Factors

During a sanitation inspection, use the following critical factors:

1. Check adequacy of firm's controls and review records covering the receipt of tuna fish. Ascertain if only tuna below the mercury guidelines and not decomposed is processed. Determine disposition of decomposed or over tolerance tuna.
2. Conduct organoleptic analysis of incoming tuna and of tuna being processed.
3. Check food additives to determine that only those permitted by the standards are used.
4. Check usage of insecticides and rodenticides to determine that they are used properly and do not become incidental food additives.
5. Study controls over the canning operation to assure that only good quality tuna is canned and that it is canned in accordance with FDA requirements.

88.3.2 Raw Materials

1. Determine adequacy of firm's controls for assuring that they are not canning decomposed tuna or tuna with excessive mercury.
2. Determine disposition of lots of tuna which are rejected because of excessive mercury.
3. Review firm's assay records and controls regarding mercury analysis of raw, in-process, and finished canned tuna.
4. Ascertain adequacy of controls firm utilizes to assure that the species of tuna canned are those allowed by standards.
5. Conduct organoleptic analysis of incoming raw tuna, frozen tuna which has been thawed for canning, and of any tuna being held for excessively long periods at room temperature.
6. Determine disposition of any tuna which is found to be decomposed (destruction, diversion, etc.).
7. Check raw material storage area for presence of insects, rodents, or other possible contaminants.
8. Check food additives in storage to ascertain if they are allowed in canned tuna as per 21 CFR 161.190(a) – Canned Tuna Standards.
9. Check firm's storage of rodenticides and insecticides to determine that they are used in accordance with instructions and are not becoming secondary food additivese.

88.3.3 Processing

1. Check firm's can seamers to determine if they are functioning properly.
2. Determine adequacy of firm's check on can seaming.
3. Determine if firm's retorts or continuous cookers are functioning properly.
4. Review recording charts from retorts and continuous cookers to ascertain if tuna was processed at proper time and temperature relationship.
5. Determine firm's postprocessing can handling. How cans are cooled, and whether water is clean and chlorinated.

6. Examine fish at critical points in the processing procedure, for organoleptic quality such as:
   a. In butchering state–prior to precook;
   b. After precook before being canned (no long holding time after precook);
   c. After any period the tuna has been held excessively long at room temperature.
   d. Evaluate firm's canning operation for compliance with the GMPR's for low acid foods (21 CFR 113).
   e. Check plant for proper screening and rodent proofing to eliminate insects and/or rodents.

88.3.4 Sanitation

Check:

1. Firm's operation for compliance with GMPR's Human Foods (Sanitation) 21 CFR 110.
2. Firm's equipment cleaning and sanitizing operation and determine its effectiveness.
3. If adequate hand washing and sanitizing facilities have been provided and that signs are posted directing employees to use them.
4. Employees use of hand sanitizing solutions and whether solutions are maintained at proper strength.
5. Firm's usage of insecticides and rodenticides so they do become incidental food additives.
6. Freezers for proper storage temperatures and for sanitary storage.
7. Review firm's records regarding assay of finished product for mercury, decomposition, and other quality factors.
8. Review firm's assay records to determine if the canned tuna complies with the Standard (21 CFR 161.190).
9. Ascertain if the food additives used are permitted by the Standards and other legal requirements.

88.4 OYSTERS

Most oyster shucking operations are handled by state inspection agencies. For procedures see FDA standard guidelines on interstate shellfish sanitation. Microbiological considerations are of prime importance in any shellfish gathering and processing plant. Time-temperature abuses enter into most problems with the products. However, the high value of these products has made economic violations even more profitable to the unethical operator. During an evaluation of sanitation, use the critical factors as follows:

1. Check for evidence of contamination from the presence of cats, dogs, birds, or vermin in the plant.
2. Check results of any testing conducted on incoming oysters including filth, decomposition, pesticides, or bacteria.

3. Check for possible incorporation of excessive fresh water through (a) prolonged contact with water or (b) by insufficient drainage.
4. Determine if employee sanitation practices preclude adding contamination (clean dress and proper use of 100 ppm chlorine equivalent hand sanitizers).
5. Determine if equipment is washed and sanitized about every 2 h.
6. Check for time-temperature abuses that may cause rapid bacterial growth.

88.5 BLUE CRAB (FRESH AND PASTEURIZED)

88.5.1 Sanitation Critical Factors

During a sanitation evaluation, use critical factors as follows:

1. Check for evidence of contamination from rodents, insects, flies, birds, and domestic pets.
2. Determine if employee sanitation practices preclude adding contamination (clean dress and proper use of 100 ppm chlorine equivalent hand sanitizers) particularly during pick out of shells from crabmeat.
3. Determine if equipment is washed and sanitized about every 2 h.
4. Check for time-temperature abuses which may cause rapid bacterial growth.
5. Check testing of incoming crabs for decomposition, bacterial load, pesticides, and dead crab removal prior to processing.
6. Check firm's usage of rodenticides and insecticides to determine that they do not contaminate the in-process crabs.

Let us look at the sanitation aspects of the different stages of operation.

88.5.2 Raw Materials

1. Check receiving and handling process prior to cooking.
2. See if firm discards all dead crabs prior to cooking. If not estimate percentage of dead crabs utilized.
3. Note any rodent or insect activity in the receiving area.
4. If the firm refrigerates the live crabs prior to cooking, see if they are kept in a separate cooler from the processed crabs.
5. Check results of any testing of incoming crabs including bacteriological results and pesticides.

88.5.3 Manufacturing Process

To evaluate the sanitation of the manufacturing process, check on the following:

88.5.3.1 Cooking. Check product flow and determine time and temperature of cooking and type of cooker.

1. Retort.
2. Live steam. Check boiler compound used.

3. Review recording charts for retorts.
4. Determine venting procedures.

88.5.3.2 Cooling. Check time and temperature relationship and:

1. How long cooked crabs are held at room temperature.
2. Any processing delays between cooking, cooling, and picking.
3. Whether cooled crabs are refrigerated until picked.
4. Whether cooked crabs are stored in same baskets as cooked in or are transferred to another container.
5. If refrigerator is used for storing cooled crabs, it is used only for this purpose.

88.5.3.3 Picking. Check on the following sanitation aspects:

1. If picking table is cleaned and sanitized prior to use, at appropriate times during the day, and at the end of the day.
2. If the picking table is not cleaned and sanitized between each new supply of crabs, if all crabs on the table are picked prior to the addition of new crabs. Check handling of crab claws prior to picking.
3. Pickers hands for cuts, sores, and so on.
4. That picking utensils are of proper construction.
   a. See if all metal knives without wooden handles are used.
   b. Check to see that the workers do not wrap the handles of the knives with paper towels, cloth, or string.
   c. See if all stainless steel or other metal shovels with steel handles and shafts are used for placing the crabs onto the picking table. Check shovel storage and see whether it is used for anything besides crabs.
5. If claws are picked mechanically, obtain procedure and check operation.
6. Check on how often pickers deliver the picked meat to the packing room.

88.5.3.4 Packing

1. See if picked crab meat is placed directly into the can or into holding pans. If the crab is "deboned" prior to packing, check on how long it is held.
2. See if weighed crabmeat weighed into final can is closed and iced at frequent intervals. Determine if pickers do their own weighing and final packing.
3. Check on how finished packaged crab meat is stored or if it is shipped the same day it is packaged.
4. See if ice used is from an approved source. Check storage of ice.

88.5.3.5 Pasteurization

1. Check the can closing system and can handling prior to pasteurization.
2. Check time-temperature of pasteurization process.
3. Check on how pasteurized cans are cooled and stored.
4. See if the finished canned crabmeat is stored in a refrigerator prior to shipping and how long it is held prior to shipment.
5. Determine shipping operation: refrigerated trucks iced baskets, and so on.

88.5.3.6 Lighting, Ventilation, Refrigeration, Equipment

1. Determine if building is adequately lighted and ventilated.
2. Check if the cooling and refrigerating facilities are adequate to do the job.
3. See if equipment is of proper construction.

88.5.4 Overall Sanitation

1. See if the building provides for a separation of the various processes.
2. See if building is so constructed to be free from rodent or insect entry points or harborages and whether there are rodents or insects in plant.
3. Check if product contact surfaces (tables, carts, pans, knives, etc.) are of proper construction. See if seams are sealed to avoid product buildup.
4. Obtain in detail the firm's plant and equipment cleaning and sanitizing procedures and check if all equipment is cleaned and sanitized as necessary.
5. Determine if employee toilets and hand-washing facilities are provided, maintained, and supplied and if handwashing facilities are located in various processing areas.
6. Determine if hand sanitizing solutions are provided at appropriate locations, maintained at proper levels at all times, and used when necessary. Check hand-sanitizing solutions, strength at various intervals during the inspection. Check to see if employees use hand dips when necessary.
7. Evaluate the firm's operations and employee practices for compliance with the Human Food (Sanitation) GMPR's, CFR part 110 and the Food Code.
8. Document any insanitary conditions noted that could lead to the contamination of the firm's crabs ox crabmeat with filth and/or bacteria.
9. Check storage and disposal of solid waste, for example, shells.

88.5.5 Checklist for Crustacea Processor

Use the following to obtain the information necessary to make a valid assessment of the sanitation of a processor's operation.

Receiving (unload)	1. Determine condition (acceptable or decomposed)
Control aspect	2. Separate work area
Sorting	1. Remove miscellaneous species of incidental fish
Control aspect	2. Further determination of condition (quality)
Age	1. Sanitation
Control aspect	2. Time/temperature
Peeling (mechanical,	1. Sanitation
types (Model A)	2. Potable water
(PCA-1.5″ cook)	3. Separate work area (for peeling, washers,
(choice for freezing)	separators, and, if applicable, shaker-blower)

Washers	1. Sanitation
Control aspect	2. Potable water
	3. Shell and debris removal (quality)
Shaker-blower (options)	1. Sanitation
Control aspect	2. Shell removal (quality)
In-house inspection	1. Sanitation
Control aspect	2. Shell removal.
	3. Separate work area for freezing
Size graded (machine or manual)	Sanitation
Package (cans or plastic)	Sanitation
	Personnel sanitation
Control aspect	Suitable packaging materials
	Time/temperature
	Separate work area
Freeze	Time/temperature (quality)
Control aspect	

88.6 SCALLOPS

88.6.1 Background Information

The scallop industry encompasses three primary species.

1. Sea scallops
2. Bay scallops
3. Calico scallops

The processing of sea scallops is accomplished on board the vessel actually harvesting the product. Boats which process sea scallops remain at sea from 3 to 12 days depending on area and catch. In most cases, the calico scallops are harvested daily and processed at shore processing plants rather than on board the vessel. The trend, however, is toward on-board processing for this species also. Bay scallops pose a unique problem in that they may be processed in a commercial plant or at home.

88.6.2 Sanitation Critical Factors

During the evaluation of food plant sanitation, use the following critical factors:

1. Check for evidence of contamination from rodents, insects, birds, or from domestic animals.
2. Determine if equipment is washed and sanitized about every 2 h.
3. Check for time-temperature abuses which could cause rapid bacterial growth and/or decomposition.

4. Determine if employee practices preclude the addition of contaminants. Clean dress and proper use of 100 ppm chlorine equivalent hand sanitizers.
5. Determine method of icing or freezing of the scallops.
6. Ascertain if incoming scallops are tested for bacterial load, decomposition, pesticides, and so on. Review results of these tests.
7. Check usage of pesticides and rodentcides by firm, to ascertain that they do not become incidental food additives.

88.6.3 Raw Materials

Determine:

1. Geographical area where the scallops are harvested.
2. Type of scallops harvested and processed by common or species name.
3. How scallops are handled between harvesting and processing.

88.6.4 Processing

1. Observe in detail the scallop processing operation. Make a flow-plan.
2. Check shucking and evisceration process, and see if this process is physically separated from the packaging and other operations.
3. Determine source of water used in the scallop washing and rinsing operations. If treated by the processor, determine nature and extent of treatment.
4. See if equipment used in processing operation is of proper construction and design.
5. Check firm's equipment cleaning and sanitizing operation.
6. Determine time and temperature of processing operation. Check:
   a. How long between harvest and chucking and the temperature of the scallops;
   b. How long scallops are held at ambient air temperature and determine the ambient temperature;
   c. How long between shucking and rinsing and the temperature of the scallops;
   d. After being iced, how long before scallops reach an internal temperature below 40°F.
7. Check finished product packaging.
8. Determine source of ice used in icing operation and if bagged ice is used, source and: type of bag; condition of bags; conditions of storage.
9. Check finished product storage facilities and condition.
10. Check on the use of any food additives to determine if used at allowable levels.

88.6.5 Overall Sanitation

1. See if building or vessel is free from rodent or insect activity.
2. Check that toilets and hand-washing facilities provided are properly located and maintained.
3. Determine strength and type of hand-sanitizing solutions used and the sanitizer's location.

4. Note any employee practices that could lead to the contamination of the scallops with filth and/or bacteria.
5. See if water and ice used in the process is from an approved source and list source.
6. Determine method of shell and waste material disposal.
7. Evaluate the firm's operation for compliance with the Human Foods (Sanitation) CGMPR's,21 CFR 110 and the Food Code.
8. Document any insanitary conditions noted which could lead to the contamination of this firm's products with filth and/or bacteria.

88.7 SHRIMPS

88.7.1 Sanitation Critical Factors

Breading of shrimp has long posed a problem from an economic standpoint. In addition, the time-temperature abuses present a great potential for food poisoning organisms. The growing scarcity and consequential high value of the raw material make the breading standards even more important. Review Breaded Shrimp Standards (21 CFR 161) prior to evaluate plant sanitation.

During a sanitation assessment, use critical factors as follows:

1. Check for the presence of cats, dogs, birds, or vermin in the plant.
2. Review testing of incoming shrimp. Check results of tests for decomposition, bacterial load, pesticides, and other possible adulterants.
3. Evaluate operation for compliance with 21CFR 12.1- Raw Breaded Shrimp.
4. Watch for any time-temperature abuses in the handling of seafood.
5. Determine that employee hygienic practices are satisfactory, for example, clean dress, washing of hands, and use of 100 ppm chlorine equivalent hand sanitizers.
6. Note any equipment defects which cause seafood to lodge, decompose, then dislodge into the pack.
7. Observe breading operations for suspected excesses (21 CFR 161.175/6), lack of coolant to keep batter mix below 50°F in an open system and below 40°F in closed system.

Note the misuse of pesticides, abuse of color or food additives, deviations from standards, and so on.

88.7.2 Raw Materials: Receipt and Storage

Determine if:

1. Shrimp and other raw materials are inspected upon receipt for decomposition, microbial load, pesticides, and filth.
2. Raw materials susceptible to microbial contamination are received under a supplier's guarantee. Raw material specifications exist and only wholesome raw materials are accepted into active inventory. Determine disposition of rejected raw materials.

3. Shrimp receiving and storage facilities are physically adequate.
4. Frozen shrimp are stored at 0°F (−18°C) or below.
5. Fresh or partially processed shrimp are iced or otherwise refrigerated to maintain a temperature of 40°F (4°C) or below until they are ready to be processed.
6. Decomposed shrimp are being processed.
   a. Examine shrimp as received, and again after sorting, for decomposition. Classify as passable (class 1), decomposed (class 2), or advanced decomposition (class 3). Less experienced inspectional personnel should submit some of class 2 and class 3 shrimp for confirmation by the laboratory.
   b. Prompt handling and adequate sorting is necessary to prevent decomposition. Check times and temperatures.
   c. Where decomposed shrimp are going into canned or cooked-peeled shrimp, collect investigational samples of the finished pack. Give attention to disposition of loads showing a high percentage of decomposition which cannot be adequately sorted, and to disposition of reject shrimp. Make certain that "bait shrimp" is denatured.

1. Fresh raw shrimp are washed and chilled to 40°F (4°C) or below within two hours of receipt. Frozen shrimp should be held at 0°F (−18°C) or below. Determine if they are examined organoleptically when received.
2. Peeled and deveined shrimp are promptly chilled to 40°F (4°F) or below.

88.7.3 Plant Sanitation

Determine if:

1. The water (ice) is:
   a. From an approved source;
   b. Disinfected and contains residual chlorine;
   c. Sampled and analyzed for contamination;
   d. Handled in a sanitary manner.
2. Drainage facilities are adequate to accommodate all seepage and wash water.
3. The plant has readily cleanable floors which are sloped and equipped with trap drains.
4. The plant is free of the presence of vermin, dogs, cats, or birds.
5. The screening and fly control is adequate.
6. Offal, debris, refuse is placed in covered containers and removed at least daily or continuously.
7. Adequate hand-washing and sanitizing facilities are located in processing area and are easily accessible to the preparation, peeling, and subsequent processing operations.
8. Signs are posted directing employees handling shrimp and other raw materials to wash and sanitize their hands after each absence from the work station.
9. Employees actually wash and sanitize their hands as necessary (before starting work, after absences from the work station, when hands become soiled, etc.).

10. Hand-sanitizing solutions are maintained at 100 ppm available chlorine or the equivalent and are used.
11. Persons handling food or food contact surfaces wear clean outer garments, maintain a high degree of personal cleanliness, and conform to good hygenic practices.
12. Management prevents any person known to be affected with boils, sores, infected wounds, or other sources of microbiological contamination from working in any capacity in which there is a reasonable probability of contaminating the food.
13. The product is processed to prevent contamination by exposure to areas involved in earlier processing steps, refuse, or other objectionable conditions or areas.
14. Food contact surfaces are constructed of metal or other readily cleanable materials.
15. Seams are smoothly bonded to prevent accumulation of shrimp, shrimp material, or other debris.
16. Each freezer and cold storage compartment used for raw materials, in process or finished product is fitted with required temperature indicating devices.
17. Unenclosed batter application equipment is flushed and sanitized at least every 4 h during plant operations and all batter application equipment is cleaned and sanitized at the end of and the beginning of the days operation.
18. Breading application equipment and utensils are thoroughly cleaned and sanitized at the end of the days operations.
19. Utensils used in processing and product contact surfaces of equipment are thoroughly cleaned and sanitized at least every 4 h during operation.
20. All utensils and product contact surfaces excluding breading application equipment and utensils are rinsed and sanitized before beginning the days operation.
21. Containers used to convey or store food are handled in a manner to preclude direct or indirect contamination of the contents.
22. The nesting of containers is prohibited.

88.7.4 Processing

Determine if:

1. Raw frozen shrimp are defrosted at recommended temperatures (air defrosting at 45°F (7°C) or below, Or in running water at 70°F (21°C) or below in less than 2 h).
2. Fresh raw shrimp are washed in clean potable water and chilled to 40°F (4°C) or below.
3. Fresh shrimp are adequately washed, culled, and inspected.
4. Every lot of shrimp that has been partially processed in another plant, including frozen shrimp, is inspected for wholesomeness and cleanliness.
5. Shrimp entering thaw tank are free from exterior packaging material and substantially free of liner material.
6. On removal from thaw tank. shrimp are washed with a vigorous potable water spray.
7. Shrimp are removed from thaw tank within 30 min after they are thawed.

8. During the grading, sizing, or peeling operation the:
   a. Equipment is cleaned and sanitized before use;
   b. Water is maintained at proper chemical strength and temperature;
   c. Raw materials are protected from contamination.
9. Sanitary drainage is provided to remove liquid waste from peeling tables.
10. Firm prohibits the practice of salvaging shrimp (i.e., repicking the accumulated hulls and shells for missed shrimp or shrimp pieces).
11. Peeled and deveined shrimp are promptly chilled to 40°F (4°F) or below.
12. Peeled shrimp are transported from peeling machines or tables immediately, or, if containerized, within 20 min.
13. Peeled shrimp containers, if applicable, are cleaned and sanitized as often as necessary, but in no case less frequently than every 3 h.
14. When a peeler is absent from his duty post, his container is cleaned and sanitized prior to resuming peeling.
15. Peeled shrimp that are transported from one building to another are properly iced or refrigerated, covered, and protected.
16. Shrimp are handled minimally and protected from contamination.
17. Shrimp which drop off processing line are discarded or reclaimed.
18. Shrimp are washed with a low-velocity spray or in unrecirculated flowing water at 50°F (10°C) or below just prior to the initial batter or breading application, whichever comes first, except in cases where a predust application is included in the process.
19. Removal of batter or breading mixes or other dry ingredients from multiwalled bags is accomplished in an acceptable manner.
20. Batter in enclosed equipment is assured a temperature of not more than 40°F (4°C) and disposed of at the end of each work day, but in no circumstances less often than every 12 h.
21. Batter in an unenclosed system is maintained at or below 50°F (10°C) and disposed of at least every 4 h and at the end of the days operation.
22. Breading reused during a day's operation is sifted through a 1/2 in. or smaller mesh screen.
23. Breading remaining in the breading application equipment at the end of a days operation is reused within 20 h and is sifted as above and stored in a freezer in a covered sanitary manner.
24. Hand batter pans are cleaned, sanitized and rinsed between each filling with batter or breading.

88.7.5 Finished Product Process and Quality Assurance

Determine if:

1. Processing and handling of finished product is:
   a. Performed in a sanitary manner;
   b. Protected from contamination;
   c. Arranged to facilitate rapid freezing.

2. Manual manipulation of breaded shrimp is kept to a minimum.
3. Aggregate processing time, excluding the time required for thawing frozen material, is less than 2 h, exclusive of iced or refrigerated storage time.
4. Breaded shrimp are placed into freezer within 30 min of packaging.
5. Breaded shrimp are frozen in a plate or blast freezer at -20°F (-29°C) or below.
6. Storage freezer is maintained at or below 0°F (-18°C).
7. In-line, environmental, and finished product samples are analyzed and evaluated at least weekly for microbial conditions. Review these analytical records if available.
8. Firm has established microbiological specifications for the final product. If so, review and report these specifications.
9. Firm withholds from distribution lots which do not meet their established microbiological standards.
10. Finished product is handled and stored in a manner which precludes contamination.
11. Labels bear a cautionary statement to keep product frozen.

88.8 SMOKED FISH

88.8.1 Sanitation Critical Factors

During an evaluation of the sanitation of a smoking fish operation, use critical factors as follows:

1. Check sanitary conditions under which firm is operating, including any evidence of insanitation and contamination associated with insects, rodents, microorganisms, chemicals, or other possible sources. Check raw material and packaging material storage areas as well as other susceptible locations in the plant.
2. Review raw material receiving records for DDT and other pesticides, decomposition, and bacteriological quality.
3. Check food and color additives to ascertain that they are allowed for use and are being used properly.
4. Observe employee practices to make sure that they are not acting as routes of contamination.
5. Ascertain if the various operations including raw material receipt and storage, defrosting, brining, and so on, are acceptable.
6. Review recording charts to ascertain what time/temperatures of smoking have been, this may vary depending on the desired salt content the firm is trying to achieve.
7. Check finished stored product (i.e., any smoked chubs in which nitrite is used) to ascertain the internal temperature based on the time since smoking. (Temperature within 3 h of cooking and again within 12 h of cooking.)

88.8.2 Plant Sanitation and Facilities

1. Check method(s) for cleaning and sanitizing utensils, conveyors, smoking racks, and other food-contact surfaces used in daily operations.

2. Check the strength and adequacy of hand-sanitizing and equipment-sanitizing solutions. The minimum effective chlorine concentration for hand-sanitizing solutions is 100 ppm and for equipment-sanitizing solutions 200 ppm. Iodine solutions should be 15 ppm for hand-sanitizing solutions and 25 ppm for equipment-sanitizing solutions. Determine if maintained at proper levels.
3. Determine method used to separate finished product cooling, packaging, and storage areas from the uncooked product and processing areas.
4. Determine the adequacy of plant waste disposal operations.
5. Check if hand-washing, toilet, and sanitizing facilities have been provided and that signs have been posted directing the employees to wash and sanitize hands following use.

88.8.3 Raw Materials

Determine:

1. Source (area and distributor) and species of fish processed by the firm including the type selected for full coverage during this inspection.
2. Process condition in which bulk fish is supplied (e.g., fresh, frozen, mild cured, brined, etc.).
3. Quality of fish received. Organoleptic examination should be performed and results reported.
4. Raw fish handling procedures (e.g., defrosting, draining procedures encountered).
5. Available chlorine or iodine concentrations in hand dip or equipment sanitizing solutions, if used.
6. Time/temperature intervals for each step in the raw fish handling operations.
7. If incoming fish are sampled and analyzed for the presence of DDT and other pesticides.

88.8.4 Processing

88.8.4.1 Salting and Brining. Determine:

1. Size of fish or pieces of fish brined noting variations of fish size and sizing procedures.
2. Form and grade of salt (NaCl) used in the brining.
3. Ratio of brine to fish. Determine actual or near estimates of weight of salt, volume of water and weight of fish being brined.
4. Concentrations of brine (NaCl) solutions in degrees Salinometer at the initiation of brining, during brining, and at the conclusion of the brining operation. A reduction in salt concentration in the brining solution after brining may indicate salt uptake by the fish during brining. [CAUTION: If Salinometers are made of glass, the degree of salinity should be read in a plastic graduate. DO NOT put the salinometer directly into the tank with fish. It could break and contaminate the fish with glass.]
5. Time/temperatures of brining solutions at different intervals during the brining process. Include total brining time.
6. Method of agitation of brine solution during brining, if employed, noting number of times agitated and length of each agitation.

88.8.4.2 Heating, Cooking, and Smoking Operation

1. Check equipment used during heating, cooking, and smoking operation. Include oven type, source of heat, type of smoke generators, product temperature monitoring equipment, humidity regulators, and so on. (Temperature recording devices should have an accuracy of $\pm 2^{\circ}$F.).
2. Determine the methods and procedures used in drying, cooking, and smoking. Include time/temperature data, results of temperature monitoring by the firm, location of their temperature probes, and product rotation practices.

88.8.4.3 Cooling

1. Monitor time/temperature relationships during cooling to determine how long it takes to reach an internal temperature of 38°F.
2. Determine method of cooling.
3. Check observable procedures and conditions which can contribute to the microbiological contamination of the processed fish. Include observations such as extended cooling time and optimum incubation temperature, exposure to airborne contamination, improper handling, or poor in-process storage conditions.
4. Determine if firm separates cooling facilities from raw processing and cooking operations.

88.8.4.4 Packaging. Determine method and types of packing including:

1. Time/temperature relationships during any use of additives or prepackaging additive treatment. Include name of quantity added, method of application, and so on.
2. Observable practices and conditions which can contribute to the microbiological contamination of the processed fish. Include lack of required facilities, excessive product handling, improper storage, and so on.

88.8.5 Storage and Distribution

1. Check type of equipment used for determining, recording, and maintaining storage temperatures.
2. Determine actual storage compartment temperatures. Refrigerated storage temperatures should be 38°F or below.
3. Determine method of distribution (e.g., refrigerated, iced, frozen, etc.).

88.8.6 Laboratory Controls

Check or determine:

1. Method and frequency of sampling. Salinity testing operations and evaluate testing procedures and frequency. Microbiological testing of processed fish, how often, methods used, adequacy of testing, and so on.
2. Checks made on in-process controls and laboratory equipment.
3. Use of outside laboratories, consultants, and so on. Include name, location, and tests each firm performs and how often tests are conducted.
4. Results of analysis from previous lots.

88.8.7 Overall Sanitation

1. Evaluate the firms operation for compliance with 21 CFR 110-GMPR's Human Foods (Sanitation).
2. Evaluate the firm's cleaning and sanitizing procedures.
3. Check if adequate handwashing and sanitizing facilities are provided and if signs directing their use are provided. Evaluate the employees use of hand dips and if they are used when necessary.
4. See if hand dips and equipment sanitizing solutions are maintained at the proper level and changed when necessary.

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89

Sustainable Intensive Aquaculture

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89.1 Introduction 969
89.2 Intensive Aquaculture Systems 971
89.2.1 Salmonids 974
89.2.2 Marine Finfish 977
89.3 Summary 979
References 979

89.1 INTRODUCTION

Aquaculture has existed for several thousands of years and in many different forms (Beveridge and Little 2002). It is hugely diverse and involves the production of both aquatic animals and plants in fresh, brackish, and marine waters from near polar to tropical environments. It has the longest history in China where earthen pond culture of common carp was recorded over 3000 years ago (Landau 1991; Li 1994). Polyculture of Chinese carps and other fish species in ponds still accounts for much of the World's aquaculture production and is a tribute to the methods developed by practitioners over thousands of years as well as to recent technological advances. A key characteristic of polyculture, partly explained by the name, is that several species are farmed together in the same body of water. This is possible because the different fish species exploit different food sources within the pond and because feeding activity and resultant fish wastes return inorganic and organic nutrients that stimulate production of plankton at the base of a

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pond's food web. Depending on the level of management and inputs into the pond, this type of aquaculture is described as extensive or semi-intensive. Within the last half of the 20th century a new approach has emerged and shifted aquaculture into an industrial phase based on the intensive monoculture of a few high value species. Intensive aquaculture is well established for several salmonid species, including rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), as well as for channel catfish (*Ictalurus punctatus*) and increasing numbers of marine finfish such as European sea bass (*Dicentrachus labrax*) and gilthead sea bream (*Sparus aurata*). All types of aquaculture continue to expand as does their share of the global fishery, this share has increased from less than 4% in 1970 to 32% in 2003 (FAO 2004). In 2003 aquaculture of finfish, and of molluscs and crustaceans (shellfish) produced 41.9 million tonnes, finfish accounted for approximately half with crustaceans and molluscs accounting equally for the majority of the remainder (FAO 2004). Although aquaculture production is based on at least 210 different plant and animal species, the majority comes from a few groups and mostly from extensive and semi-intensive farming (FAO 2002). Carp dominate aquaculture production and nine of the top 20 aquaculture species in 2000 were carp or other finfish species used in pond based polyculture (Fig. 89.1). Monetary value reflects the socioeconomic and technical basis of a particular aquaculture sector so that of the top 20 species, prawns and salmonids had a larger share of total value than they did of production (Table 89.1). In 2003, nine countries produced more than half a million tonnes of aquaculture product, including plants: China (27.7 million tonnes) dominated, India (2.2 million tonnes), Indonesia (0.9 million tones) and Bangladesh (0.8 million tonnes) were the next largest (FAO 2004). These countries used mainly semi-intensive methods where as Japan, Norway, and Chile used mainly intensive methods to produce over 0.5 million tonnes each. Aquaculture has great potential to expand further and to meet an increasing human demand for protein but to do this there may have to be dramatic changes in current structures and practice, the debate on sustainable aquaculture is only just beginning (Bardach 1997; Naylor and others 2000; Tidwell and Allan 2001; Costa-Pierce 2002). There are many ways to farm finfish and aquaculture is very broadly divided into extensive and intensive forms. It has been proposed that whilst there is a continuum from extensive to semi-intensive systems intensive aquaculture is distinct. This is primarily because, under intensive aquaculture, water provides physiological support for the fish but all

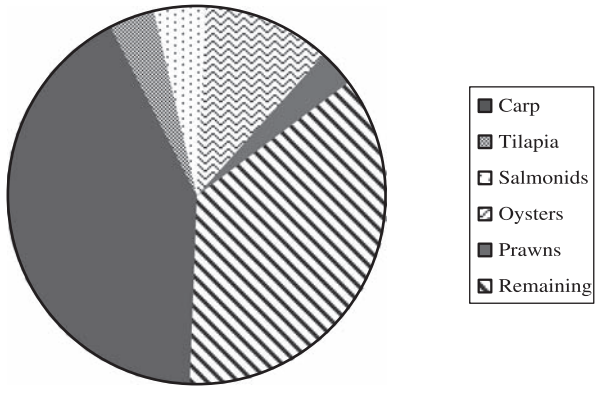

Figure 89.1 *Distribution (% of total production weight) of major aquaculture groups in 2000 (FAO 2002). Categories detailed in Table 1.*

TABLE 89.1 Major Aquaculture Species Ranked by Production in 2000.

Group	Name (US$ rank)[b]	Scientific Name	Production (Million Tonnes)
Bivalve	Pacific oyster (2)	*Crassostrea gigas*	3.94
Carp (Chinese major)	Silver carp (3)	*Hypophthalmichthys molitrix*	3.47
Carp (Chinese major)	Grass carp (4)	*Ctenopharyngodon idella*	3.45
Carp	Common carp (5)	*Cyprinus carpio*	2.72
Other (bivalve)	Small neck clam (7)	*Ruditapes philippinarum*	1.69
Carp (Chinese major)	Bighead carp (10)	*Hypophthalmichthys nobilis*	1.64
Carp	Crucian carp (13)	*Carassius carassius*	1.38
Other (bivalve)	Giant ezo scallop (8)	*Patinopecten yessoensis*	1.13
Other (finfish)	Tilapia (9)	*Oreochromis niloticus*	1.05
Salmonid	Atlantic salmon* (6)	*Salmo salar*	0.88
Carp (Indian major)	Rohu (9)	*Labeo rohita*	0.80
Carp (Indian major)	Catla (15)	*Catla catla*	0.65
Carp (Indian major)	Mrigal (17)	*Cirrhinus mrigala*	0.57
Prawn	Tiger prawn (1)	*Penaeus monodon*	0.57
Other (finfish)	Bream (16)	*Parabramis pekinensis*	0.51
Other (finfish)	Milk fish (14)	*Chanos chanos*	0.46
Other (bivalve)	Blue mussel (19)	*Mytilus edulis*	0.46
Salmonid	Rainbow trout* (11)	*Oncorhynchus mykiss*	0.49
Other (bivalve)	Clam (20)	*Anadara granosa*	0.29
Other (finfish)	Channel catfish[a] (18)	*Ictalurus punctatus*	0.27

[a]Farmed Intensively. [b]Rank within top-20 production by US$.
Source: FAO (2002).

other means of sustaining life are provided from external sources, the most obvious being the supply of nutritionally complete feeds. The aim of this chapter is to provide an overview of the range of finfish species and systems used in intensive aquaculture.

89.2 INTENSIVE AQUACULTURE SYSTEMS

The principles that drive intensive aquaculture are based around maximizing production in the shortest length of time. Intensive aquaculture is most often based on monoculture of high value and usually carnivorous species in developed countries. Fish at high densities are contained in a wide variety of structures including net-cages, tanks, raceways, and earthen ponds (Fig. 89.2). Nutritionally complete compound feeds are fed at maximum rations, and usually manufactured at specialized feed mills as pellets containing a large variety of ingredients. Arguably, for a species to be an aquaculture species the lifecycle should be closed so that it is bred under culture and all stages of its lifecycle are farmed. This is the case for salmonids (salmon and trout), cyprinids (carp), and catfish as well as for increasing numbers of marine finfish. Reproduction and propagation of major freshwater (carps and catfish) and anadromous (salmonids) farmed finfish is relatively straightforward and achieved with a high success rate. One important characteristic of these finfish is the large size and robust nature of their eggs and the ease of feeding compound feeds immediately, or soon after, the time the larvae are able to take external foods. This is in marked contrast to marine species which have far smaller eggs and larvae, consequently first-feeding larvae are not fed on compound feeds and far greater investments in

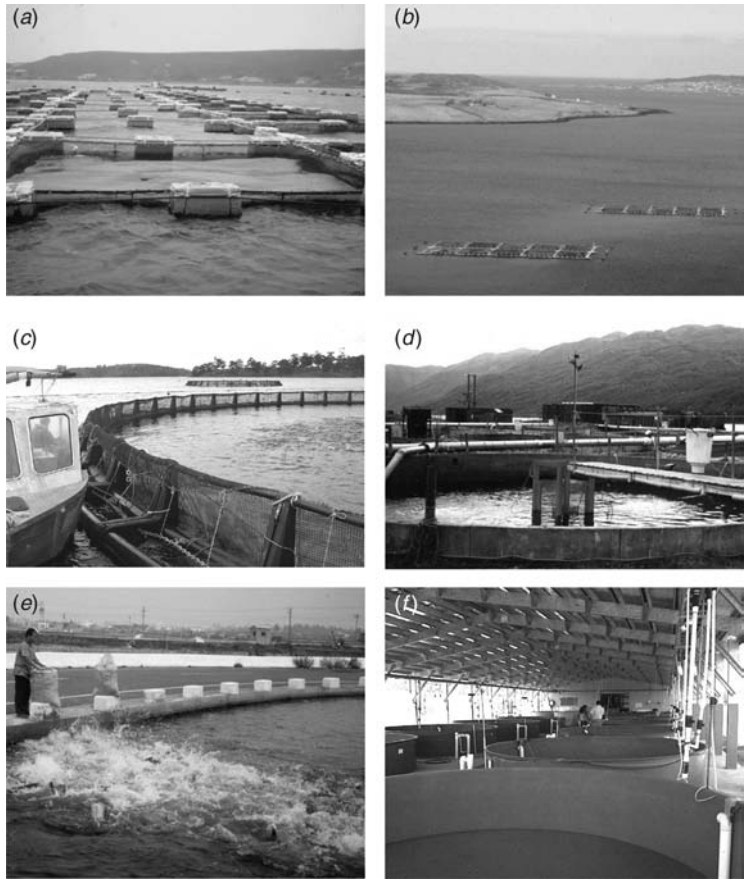

Figure 89.2 *Different forms of intensive aquaculture (a) small 4 m square-cages for grouper farming in Taiwan, (b) 24 m square-cages for Atlantic salmon farming in Scotland, (c) ring-cages for Atlantic salmon farming in Tasmania, (d) concrete tanks for turbot in Scotland, (e) road-side ponds for cobia farming in Taiwan (f) tanks used for intensive rearing of Asian sea bass fry in Australia.*

technologies for intensive larval rearing and for supplying live feeds have been made. Although, some industries may use extensive pond based production of fry as a more economic approach. Barramundi or Asian sea bass (*Lates calcarifer*) is one example of this practice, although intensive hatchery methods have been established some farms use extensive ponds to produce fry for intensive rearing and grow-out (Tucker and others 2002).

Maintaining fish health and disease control is clearly important to all sectors of aquaculture. Unfortunately, there are examples of large losses in production due to disease. The Atlantic and Pacific salmon industries have suffered from major viral (e.g., IPN, infectious pancreatic necrosis; ISA, infectious salmon anemia), bacterial (e.g., furunculosis; bacterial kidney disease), and parasitic (e.g., sea lice; ameobic gill disease) diseases. Atlantic salmon suffered from major outbreaks of furunculosis in the early 1990s that resulted in yearly losses of US$77 million, fortunately the disease has declined since 1993 due to the use of effective vaccines (Holm 1999). Vaccination against a variety of potential

pathogens is now a major management procedure in the salmon industry, over 90% of all Norwegian stocks are vaccinated by injection (Holm 1999). Vaccination is only a part of the healthcare management programs now in place. These recognize the need for trained personnel, routine monitoring and quick diagnosis; to minimize stress through appropriate stocking densities, careful and minimal handling; site rotation to break the disease cycle; cooperation between farms to ensure single-year class stocking at a location in order to prevent vertical transfer and break disease cycles (Holm 1999). Aquaculture is also vulnerable to environmental impacts that include toxic algal blooms, contamination from land run-off and disasters such as oil spills. Offshore marine finfish aquaculture is increasingly being considered as a realistic approach to make dramatic increases in aquaculture production and reduce some of these risks (Ytterland and Kvalheim 2005). It has the advantages of both avoiding and not contributing to costal pollution as well as minimizing conflicts with numerous commercial and recreational groups (Bridger 2001). Technology required for holding and servicing stocks must be far more resilient than for inshore farming and greater mechanization is needed to reduce the need for manual intervention, this technology is developing rapidly but not yet fully available (Bridger 2001; Ytterland and Kvalheim 2005). Large robust cages for offshore locations are under development for several aquaculture sectors including salmon, flatfish, sea bass, and sea bream (Lien 1993). Examples include various designs and one of the main features is that they can be fully submerged to reduce damage from wave action at the sea surface (Lien 1993; Ytterland and Kvalheim 2005). Individual units can be linked to central stations, for example, decommissioned oil and gas platforms have been under trial. There have also been significant advances in closed-water systems, these are usually indoor tank-based systems that place reliance on technology to reduce water use and maintain its quality with advanced physical and biological filtration.

Finfish nutrition and aquafeed development currently face important issues, aquafeeds use large amounts of fish meal and fish oil. Although it is argued that aquaculture has not caused any increase in fish meal production, existing production has been increasingly allocated to the aquafeed market from other feed sectors (Tidwell and Allan 2001). In the 10 years between 1988 and 1998 the proportion of the global fish meal supply used in aquafeeds increased from 10% to 35% and is predicted to increase further (Tidwell and Allan 2001). This is not sustainable and considerable research and development is focused on developing alternative protein and oil sources from various plants and microorganisms (Hardy 1996; Carter and others 2003). Experimental feeds that do not contain marine products have been developed for large channel catfish, in fact the total animal meal now used in commercial catfish feeds is less than 2% (Robinson and Li 2002). However, channel catfish is omnivorous and one of the few noncarnivorous finfish farmed intensively. The complete replacement of marine ingredients in aquafeeds for carnivorous species poses more challenges that include plant meals being low in digestible protein, high in carbohydrates, high in antinutritional factors and having poor essential amino acid profiles. For example, salmonids have limited ability to both digest and metabolise dietary carbohydrate and the optimum dietary inclusion ranges between 10–20% (Hemre and others 1995). Milled but relatively unprocessed plant meals can contain over 70% carbohydrates and less than 20% protein and this clearly restricts their inclusion level in aquafeeds. For many years soybean (*Glycine max*) meals from which oil has been extracted have proved an excellent source of digestible high quality protein for aquafeeds but other suitable highly processed ingredients manufactured from plant sources are both limited and expensive. Consequently, a number of microbial as well as plant sources are

being developed to produce ingredients that are more suited to inclusion in aquafeeds, important characteristics are higher digestible protein, a more suitable essential amino acid profile and lower levels of carbohydrate and antinutritional factors. Products made from lupins (*Lupinus* sp.), canola (*Brassica* sp.), and field peas (*Pisum* sp.) and from unicellular sources such as bacteria, yeasts and algae show considerable promise (Storebakken and others 1998; Carter and Hauler 2000; Metailler and Guillaume 2001).

Arguably, the replacement of fish oil with terrestrial oils in aquafeeds is more urgent that the replacement of fish meal. For example, Atlantic salmon aquafeeds contain a high proportion of fish oil (over 38%) and their high global production means aquafeeds are the major user of global fish oil. This level of use and the probability of increased competition for fish oil from other sectors (e.g., nutraceuticals) have the potential to severely restrict further expansion of salmon aquaculture. Animal fats are unsuitable due to the abundance of saturated fatty acids and low concentrations of unsaturated fatty acids, particularly omega-3 series (Metailler and Guillaume 2001). Fortunately, it now appears feasible to replace at least 75% of the fish oil in Atlantic salmon feeds with a blend of plant oils (Torstensen and others 2005). The majority of oils used to replacement fish oil have been of plant seed in origin such as canola (rapeseed), linseed, soybean, and sunflower (Bransden and others 2003). The major issue is that plant oils are low in the omega-3 fatty acids that confer the health benefits to humans, fish oil is rich in omega-3 fatty acids, particularly the long chain polyunsaturated omega-3 fatty acids eicosapentaenoic (EPA, 20: 5*n*-3) and docosahexaenoic (DHA, 22: 6*n*-3) acids (Sargent and others 2001; Bransden and others 2003). One approach is to look at ways of minimizing the inclusion of fish oil over the production cycle, for example, the amount of dietary fish oil may be increased towards the end of production to boost omega-3 flesh levels prior to harvest. However, fish have their own requirements for omega-3 and omega-6 fatty acids so that the correct dietary inclusion levels and balance between the different fatty acid series are required throughout production. Linseed oil, unlike other plant seed oils, is rich in long chain omega-3 fatty acids and has been investigated specifically to supply these in fish oil replacement studies (Bell 2000). Microorganisms such as thraustochytrids and marine bacteria show potential for production in large batch culture, because they can be very high in EPA and DHA they would make excellent replacements for fish oil (Lewis and others 1999; Carter and others 2003). Freshwater fish and salmonids readily convert linolenic acid (LNA) to longer chain EPA and DHA. Recently, a dietary source of stearidonic acid (SDA), a possible intermediate between LNA and EPA, was used by Atlantic salmon to increase their EPA and DHA content (Miller and others 2005). Considerable success has been achieved in replacing fish oil but further solutions are urgently required. One issue of importance in the replacement of fish meal and fish oil, particularly with plant oils has the potential to affect taste and consumer preferences. Interestingly there may be a preference for salmon flesh that is less "marine," salmon fed a blended plant oil for the entire production cycle were preferred over salmon fed entirely fish oil by a trained taste panel (Torstensen and others 2005). Encouragingly, all the fish oil could be replaced without compromising flesh quality and 75% could be replaced without compromising the long chain omega-3 composition in relation to human health (Torstensen and others 2005).

89.2.1 Salmonids

Around 10 salmonid species (members of the family Salmonidae) are farmed in 60 countries (Purser and Forteath 2003): rainbow trout, Atlantic salmon, and coho salmon

(*Oncorhynchus kisutch*) account for over 90% of production. These species are endemic to temperate seas of the northern hemisphere, *Oncorhynchus* species from the Pacific and *Salmo* species from the Atlantic. Export for use in recreational fisheries and aquaculture makes them global species with a considerable southern hemisphere presence (Willoughby 1999). Rainbow trout have been cultured for many years and Denmark pioneered pond based production of plate sized fish at the end of the 19th century (Willoughby 1999). In the last 10 years Atlantic salmon has become the major salmonid species due to a huge expansion in sea-cage farming in Norway and several other countries. Atlantic salmon are typically anadromous (return from the sea to spawn in freshwater) with freshwater and seawater phases in their lifecycle. Adults of up to 30 kg famously migrate up the rivers and streams that they last swam down as immature parr several years previously. Mature males and females return to freshwater sometime in the 12 months before they spawn, the female makes a hollow redd in a gravelly stream bed and the male fertilizes the eggs as they are released. The buried eggs hatch into a yolk-sac stage (alevin) and emerge as fry that start feeding on animal prey. The fry grow into parr that then undergo smoltification, a metamorphosis taking several months, to enable migration into the sea. After 2–5 years at sea (sea-winters) the fish return as mature adults, grilse mature early and return after only one sea-winter. Aquaculture followed this natural lifecycle but increased environmental control and manipulation, intense nutritional regimes, coupled with selective breeding, now maximize production over as much of the year as possible.

Selective breeding programs are at various stages of development throughout the world, in Norway it is estimated that 65% of farmed Atlantic salmon and rainbow trout originate from the National Breeding Program that started in 1971 (Gjedrem 2000). Growth rate and then increased time to maturation were used as key selection characteristics. More recent selection characteristics have used disease challenge to increase disease resistance and measurements of flesh color, fat content, and fat distribution to improve meat quality (Gjedrem 2000). There has also been development and testing of genetically modified or transgenic fish. Genetically modified Atlantic salmon parr containing a growth hormone transgene (chinook salmon growth hormone gene attached to an antifreeze protein promoter sequence from ocean pout) grew at over two and half times the rate of control fish due mainly to increased feed intake (Cook and others 2000).

Straightforward and effective procedures are followed to ensure high fertilization rates and efficient production of parr, ready for transfer as smolts from fresh to sea-water (Willoughby 1999). Smolts usually have to be transported from the hatchery to the grow-out site, this can be by land, air, or sea and care is taken to reduce stress. Only salmon parr that reached a minimum size during the "smoltification window" will be suitable for transfer, it is normally in the spring for Atlantic salmon but more variable for Pacific salmon. In contrast, successful sea-water transfer of rainbow trout, a nonsmolting species, is related principally to larger size (Purser and Forteath 2003). Controlling the supply of smolt enables stocking over more of the year and more even production throughout the year. This is particularly important when a large proportion of fish mature within one sea-winter as grilse which are smaller than older salmon (Purser and Forteath 2003). One-year smolt is the normal age category for smolt but half-year, one-and-a-half and two-year smolt are also produced and used (Willoughby 1999). Once in sea-water the on-growing phase is mainly in sea-cage systems, large bag-nets held on floating frames that are anchored to ensure the frames stay in the same place. Bag-nets are 5–20 m deep and their shape is maintained by devices such as metal rings around the circumference of

the net, weights, and lead-core ropes (Purser and Forteath 2003). Groups of cages are usually held and anchored together and can, depending on the design, be joined by walkways or only accessed individually by boat. Galvanized steel square-cages are typically available in 12–24 m^2 sections, a 24 m section would consist of a 15 × 15 m net surrounded by walkways with attached floatation devices (Fig. 89.2). The other main type of sea-cage is the circular ring-cage, buoyant circular ring structures are used to attach a circular bag-net, predator net, mooring cables and other ancillary structures such as bird netting or feeders (Fig. 89.2). These structures can range in circumference from 60 to over 120 m and are usually placed apart to allow greater water flow through a group of sea-cages, they are easier to manage independently of each other. There are a number of commercial variations of square- and ring-cages that may hold only a few tonnes to over 150 tonnes (Purser and Forteath 2003). Mature salmon are not desirable in a production run, mortality is higher in seawater, they partition feed resources into unwanted reproductive growth rather than somatic growth and develop many poor external and flesh quality characteristics that reduce marketability (Purser and Forteath 2003). Whether maturation occurs is highly influenced by size and nutritional status so that faster growing fish, often in warmer temperatures, will mature relatively quickly but be of a smaller size. Strategies to control the effects of maturation include the production of sterile triploid fish or the use of all female stock, since females show lower rates of maturity than males, and manipulation of photoperiod and lighting regimes (Pankhurst and Porter 2003; Porter and others 2003).

Several quality factors are considered of primary importance to the Atlantic salmon industry, the consumer emphasizes freshness, fat content, texture, and color whilst the producer also places priority on production yields (Torrissen and others 2001). The replacement of fish oils by plant oils and its effect on flesh quality have been discussed above. Two carotenoid pigments, canthaxanthin and astaxanthin, are mainly responsible for the pink coloration of salmon flesh. Species, lifecycle stage, and individual variation are key biological factors that influence carotenoid fixation in flesh. Salmonids, unlike some other aquaculture groups such as crustaceans, are unable to synthesize astaxanthin from precursors so it must be included in the diet (Choubert 2001). Lifecycle is important and salmonids have to reach a minimum size before carotenoids are retained in muscle, in addition carotenoids are remobilized from the muscle of maturing salmon and concentrated in the skin and in the orange eggs (Choubert 2001). Important factors that relate directly to aquafeeds are the pigment type, ingredient source, and dietary concentration. For example, carotenoids are lipid soluble and more digestible when contained in oils rather than in meals such as shrimp meal. Significant digestive and metabolic losses occur and carotenoids are deposited in many tissues besides the target muscle. Consequently, deposition in muscle occurs at an optimum dietary inclusion level, unfortunately, efficiency is generally less than 15% and decreases with dietary inclusion which further increases the cost of pigmentation. Crustacean meals, yeasts, and algae and other aquafeed ingredients are relatively rich in carotenoids but industrial synthesis of free forms of both canthaxanthin and astaxanthin allows more efficient inclusion in aquafeed formulations. Carotenoids are easily oxidized which means pigment sources that are protected from oxidation will be more effective. Recommended inclusion levels are 80 and 100 mg per kg of feed for canthaxanthin and astaxanthin, respectively (Choubert 2001). Pigments are very expensive and although only small amounts are used this increases the feed cost by 15–30% (Choubert 2001).

89.2.2 Marine Finfish

In many of countries that use intensive aquaculture product there is a premium on marine rather than freshwater finfish. Consequently, there is considerable interest in developing aquaculture for a variety of marine species, as yet there is no intensive aquaculture on the scale of Atlantic salmon. Good examples of intensive marine aquaculture in which the lifecycle is closed and that have high production, are European sea bass and gilthead sea bream in the Euro-Mediterranean region (Harache and Paquotte 1998), red drum (*Sciaenops ocellatus*) in the southern United States (Gatlin 2002), and red sea bream (*Pagrus major*) in Japan. Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*), cobia (*Rachycentron canadum*), and grouper species (e.g., *Epinecphelus* spp.) show potential (Kilduff and others 2002; Tucker and others 2002; Rimmer and others 2004).

In marine finfish production bottlenecks are associated with larval stages and an extensive program of research and development, that includes validation at a commercial scale, appears necessary for each species. Rotifers (*Brachionus* sp.) and brine shrimps (*Artemia* sp.) form the basis of most larval rearing protocols, rotifers are smaller and always fed first and then alongside the larger brine shrimp in order to maintain a food source for smaller individuals, and to provide a new food source for larger individuals. As the larvae grow they are weaned off rotifers and then off the brine shrimp and onto compound feeds. Other variations include "greenwater" techniques, whereby microalgae are also grown because they appear to benefit both the live feed and the finfish larvae. Live feeds are often "enriched" by feeding them commercial preparations to increase concentrations of specific nutrients, particularly polyunsaturated fatty acids and vitamins. A simple larval rearing protocol such as one for red sea bream would be microalgae (days 3–25), rotifers (days 3–33), brine shrimp (days 20–40), and compound feed (days 20–60). On a few days, 20–25, all four feeds might be available.

European sea bass production has increased steadily over the last 10 years and all aspects of production, from larval rearing to broodstock management, are well developed and commercial hatcheries produce over 180 million juveniles for sea-based on-growing of around 45,000 tonnes per year (Kaushik 2002). Propagation is based on a relatively standard larval rearing protocol and the sequence of live feeds, rotifer followed by brine shrimp, before weaning onto a compound feed (Kaushik 2002). European sea bass larvae first-feed 4 or 5 days after hatching, live feeds are gradually replaced by compound feeds and weaning is usually complete by 40 days. Recently, a compound feed has been used immediately at first-feeding and for the entire larval period. The use of ingredients that contain nutrients in highly available forms, such as phospholipids and hydrolyzed proteins, supplies essential nutrients in a form that can be readily assimilated and explains the diet's success (Kaushik 2002).

The large and important Japanese yellowtail industry produced 137,000 tonnes in 2000 (FAO 2002) but this was below production at the start of the 1990s. Farming yellowtail in Japan developed from holding wild-caught fish and intensified in the 1970s; unfortunately, a too rapid expansion and limitations due to disease and markets led to greater controls on the industry and a reduction in production (Doumenge 1990). Although hatchery based propagation is possible the Japanese industry is still largely based on small ($<$10 g) wild-caught fingerlings that are grown to 50–100 g before being sold for on-growing to reach 3 kg after 12 months (Masumoto 2002). Recently, the lifecycle of a bluefin tuna (*Thunnus* sp.) was closed, successful larval production from Pacific bluefin tuna (*T. orientalis*) that

had been grown in culture from larvae was achieved (Sawada and others 2005). Research started with the capture of juvenile tuna 8 years prior to their successful spawning in 1995 and 1996, the research program started many years before and demonstrates the complexity of closing the lifecycle of marine fish and the need for considerable economic and time investment to achieve success. Further development will be required because there may be key differences between wild-caught and domestic broodstock that could result in poor larval quality and a low success rate in the production of viable juveniles from this second generation of larvae (Sawada and others 2005). Bluefin tuna are very valuable fish and attempts at forms of aquaculture have been underway for many years in Japan, Europe, Australia, and across the continent of America (Carey and others 1984; Kaji and others 1996; Carter and others 1998; Sawada and others 2005). The few commercial tuna farms, located mainly in Australia and the Mediterranean, on-grow and recondition large wild-caught juvenile tuna to market size. In Australia, for example, juvenile southern bluefin tuna (*T. maccoyii*) shoals are caught using a purse-seine, towed back in specially designed ring-cages and transferred to moored cages for 4–8 months on-growing (Carter and others 1998). Tuna are usually 10–20 kg at catch and can treble their weight, farming increases the value of a fishery catch due to increased weight and because larger tuna are more valuable on a per kilogram basis. Tuna are largely sold into Japan where grading determines their price, grading for an individual fish relates to its external characteristics, flesh quality judged by a "tail-cut" sample of muscle, and it is also relative to the season and market conditions: the grade is notoriously difficult to quantify.

The aquaculture of Asian sea bass provides an interesting view of how one species has been highly adaptable and successful under many systems (Tucker and others 2002). Its natural range encompasses the northern Indian and tropical western Pacific Oceans and it is found from Iran to Australia (Tucker and others 2002). Although aquaculture production is relatively small (20,000 tonnes in 1999) there is great potential for a species that can be cultured using intensive and extensive methods, indoors or outdoors, and that can be grown over the full range of salinity (from fresh to sea-water) and even in brackish bore waters far from the sea and that have a wide temperature range (Katersky and Carter 2005). Barramundi are catadromous (opposite to salmon) and sea water is needed for the broodstock, eggs and the first half of the larval rearing cycle and the species is also a protandrous hermaphrodite so that individuals first mature into males (after 2 years) and then into females (after 3–5 years) (Tucker and others 2002). Barramundi are very fecund and females produce hundreds of thousands of eggs per kilogram on each spawning, males are stocked with females and the floating fertilized eggs are collected by nets or from the outflow water. There are many approaches to larval rearing that range from intensive to extensive. Intensive procedures can be carried out in small tanks using a rotifer and brine shrimp sequence, rotifers from day 2 to day 15 and brine shrimp from day 10. Production methods may also incorporate greenwater and the use of microalgae over the first part of larval rearing. Extensive procedures are carried out in large earthen ponds relying on natural plankton blooms initiated by fertilizing the pond. Juveniles are easily weaned onto compound feeds and transferred from the nursery to on-growing. In Southeast Asia this uses small ponds (0.08–2 hectares), coastal impoundments or smaller cages (1–300 m^3). In Australia on-growing may be in open freshwater ponds, small cages held in freshwater ponds, large sea-cages and, because barramundi command a high market price, indoor tanks. Production is based on plate-size fish although larger fish of around 2–3 kg for fillets or as "banquet" fish are becoming more popular.

89.3 SUMMARY

Over 200 aquatic species are cultured throughout the world and many species that are new to aquaculture will be under investigation at any one time (and often rejected after some period of research and development). In this overview I have concentrated on major finfish groups that are grown using intensive techniques. As noted in the introduction the majority of finfish are farmed using semi-intensive systems, in addition there is sizable aquaculture of other vertebrates (e.g., frogs, soft-shelled turtles), invertebrate (e.g., echinoderms, sea cucumbers), and seaweeds. Important challenges face aquaculture and ensuring it is sustainable and best practice clearly does not imply only the use of intensive aquaculture since considerable improvements can be made to all systems. Which ever species or system is appropriate more emphasis will be placed on increasing efficiency and maximizing the use of valuable resources. A broader view of resource use might encompass multiple users of a location and the environmental impact of aquaculture; disease prevention rather than control and limiting the use of chemical or antibiotic solutions, sustainable use of feed ingredients, and decreasing reliance on marine products. Global forces such as climate change will have important consequences in relation to which and where species are farmed, increased water temperatures may allow species to be grown in new regions but also end the culture of other species. Aquaculture has the capacity to make an invaluable contribution to human nutrition and it therefore vital that sustainable approaches are developed.

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Section XVII

Vegetable Products

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

90

Frozen Vegetables and Product Descriptions

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90.1 Introduction 986
90.2 Frozen Asparagus 986
90.2.1 Types 987
90.2.2 Styles 987
90.3 Frozen Lima Beans 987
90.3.1 Types 987
90.4 Frozen Beans, Speckled Butter (Lima) 987
90.5 Frozen Broccoli 988
90.5.1 Styles 988
90.6 Frozen Brussel Sprouts 988
90.7 Frozen Carrots 988
90.7.1 Styles 988
90.8 Frozen Cauliflower 989
90.8.1 Styles and Requirements 989
90.9 Frozen Corn on the Cob 989
90.9.1 Styles 989
90.9.2 Lengths 990
90.9.3 Colors of Frozen Corn-on-the-Cob 990
90.10 Frozen Leafy Greens 990
90.10.1 Types 990
90.10.2 Styles 990
90.11 Frozen Okra 990

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

90.11.1 Styles 991
90.12 Frozen Onion Rings, Breaded, Raw, or Cooked 991
90.12.1 Types 991
90.13 Frozen Peas 991
90.14 Peas, Field, and Black-Eye 992
90.15 Frozen Peppers, Sweet 992
90.15.1 Types 992
90.15.2 Styles 992
90.16 Frozen Potatoes, French Fried 992
90.16.1 Types 993
90.16.2 Styles 993
90.16.2.1 General 993
90.16.2.2 Strips 993
90.16.2.3 Slices 993
90.16.2.4 Dices 993
90.16.2.5 Rissolé 993
90.16.3 Length Designations 993
90.17 Frozen Potato, Hash Brown 994
90.17.1 Styles 994
90.18 Frozen Vegetables, Mixed 994
90.18.1 Kinds and Styles of Basic Vegetables 994
90.18.2 Recommended Proportions of Ingredients 995

90.1 INTRODUCTION

This book is not the proper forum to discuss the manufacture of every processed vegetable available in the market. However, regulatory agencies such the Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have issued some minimal criteria for each processed vegetable such as: what they are, what types and styles are available, and so on. The information in this chapter describes each available frozen vegetable product and has been modified from the product grades (USDA) and product standards (FDA). Product standards and product grades are established to achieve two objectives: assure product safety and minimize economic fraud.

The information provided here has one major objective: to remind a commercial processor what each frozen vegetable is and other applicable criteria for this particular product.

90.2 FROZEN ASPARAGUS

Frozen asparagus consists of sound and succulent fresh shoots of the asparagus plant (*Asparagus officianalis*). The product is prepared by sorting, trimming, washing, and blanching as necessary to assure a clean and wholesome product. It is then frozen and stored at temperatures necessary for preservation.

90.2.1 Types

1. Green or all-green consists of units of frozen asparagus which are typical green, light-green, or purplish-green in color.
2. Green-white consists of frozen asparagus spears and tips which have typical green, light-green, or purplish-green color to some extent but which are white in the lower portions of stalk.

90.2.2 Styles

Spears or stalks style consists of units composed of the head and adjoining portion of the shoot that are 3 inches or more in length. Tips style consists of units composed of the head and adjoining portion of the shoot that are less than 3 inches in length. Center cuts or cuts style consists of portions of shoots (with or without head material) that are cut transversely into units not less than one-half inch in length and that fail to meet the definition for cut spears or cuts and tips style.

Cut spears or cuts and tips style consists of the head and portions of the shoot cut transversely into units 2 inches or less but not less than one-half inch in length. To be considered this style head material should be present in these amounts for the respective lengths of cuts:

1. $1\frac{1}{4}$ inches or less. Not less than 18% (average), by count, of all cuts are head material.
2. Longer than $1\frac{1}{4}$ inches. Not less than 25% (average), by count, of all cuts are head material.

90.3 FROZEN LIMA BEANS

Frozen lima beans are the frozen product prepared from the clean, sound, succulent seed of the lima bean plant without soaking, by shelling, washing, blanching, and properly draining. They are then frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

90.3.1 Types

1. Thin-seeded such as Henderson, Bush, and Thorogreen varieties.
2. Thick-seeded Baby Potato such as Baby Potato, Baby Fordhook, and Evergreen. Thick-seeded, such as Fordhook variety.

90.4 FROZEN BEANS, SPECKLED BUTTER (LIMA)

Frozen speckled butter (lima) beans are the frozen product prepared from the clean, sound, freshly-vined (but not seed-dry) seed of the speckled butter (lima) bean plant (*Phaseolus limensis*). The skins of the seed are pigmented and the external colors range from variegated speckling of green, pink, red, and/or lavender to purple. The product is prepared by shelling the pods; by washing, blanching, and properly draining the seeds that have

been sorted and blended or otherwise prepared in accordance with good commercial practice. They are frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

90.5 FROZEN BROCCOLI

Frozen broccoli is the product prepared from the fresh, clean, sound stalks or shoots of the broccoli plant [*Brassica oleracea* (Italica group)] by trimming, washing, blanching, sorting, and properly draining. The product is frozen in accordance with good commercial practice and maintained at temperatures necessary for its preservation.

90.5.1 Styles

1. Spears or stalks are the head and adjoining portions of the stem, with or without attached leaves, which may range in length from 9 cm (3.5 in) to 15 cm (5.9 in). The spears or stalks may be cut longitudinally.
2. Short spears or florets are the head and adjoining portions of the stem, with or without attached leaves, which may range in length from 2.5 cm. (1 in) to 9 cm (3.5 in). Each short spear or floret must weigh more than 6 g (0.2 oz). The short spears or florets may be cut longitudinally.
3. Cut spears or short spears are cut into portions which may range in length from 2 cm (0.8 in) to 5 cm (2 in). Head material should be at least 62.5 g (2.2 oz) per 250 g (8.8 oz) and leaf material should not be more than 62.5 g (2.2 oz) per 250 g (8.8 oz).
4. Chopped spears or short spears are cut into portions which are less than 2 cm (0.8 in) in length. Head material should be at least 12.5 g (0.4 oz) per 50 g (1.8 oz) and leaf material should not be more than 12.5 g (0.4 oz) per 50 g (1.8 oz).
5. Pieces or random cut pieces are cut or chopped portions of spears or short spears or other units which do not meet the requirements for cut or chopped styles.

90.6 FROZEN BRUSSEL SPROUTS

Frozen brussel sprouts are the frozen product prepared from the clean, sound succulent heads of the Brussel sprouts plant (*Brassica oleracea* L. var. gemmifera) by trimming, washing, blanching, and properly draining. The product is frozen in accordance with good commercial practice and maintained at temperatures necessary for its preservation.

90.7 FROZEN CARROTS

Frozen carrots are the clean and sound product prepared from the fresh root of the carrot plant (*Daucus carota*) by washing, sorting, peeling, trimming, and blanching, and are frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

90.7.1 Styles

Wholes (or whole carrots) retain the approximate confirmation of a whole carrot.

Halves or halved carrots are cut longitudinally into two units.

Quarters or quartered carrots are cut longitudinally into four approximately equal units. Carrots cut longitudinally or cut longitudinally and crosswise into six or eight units approximating the size and appearance of quartered carrots are also permitted in this style.

Slices or sliced carrots are sliced transversely to the longitudinal axis.

Diced carrots consist of approximate cube-shaped units.

Double-diced carrots consist of approximate rectangular shapes that resemble the equivalent of two cube-shaped units.

Strips are carrots that consists of approximate French-cut shapes, with flat-parallel or corrugated-parallel surfaces, one-half inch or more in length.

Chips are carrots that consist of predominately small-sized units (such as less than one-half cube) and variously shaped pieces or slivers in which the longest-edge dimension approximates not more than one-half inch.

Cut carrots consist of cut units that do not conform to any of the forgoing styles.

90.8 FROZEN CAULIFLOWER

Frozen cauliflower is prepared from fresh flower heads of the cauliflower plant (*Brassica oleracea botrytis*) by trimming, washing, and blanching and is frozen and maintained at temperatures necessary for preservation of the product.

90.8.1 Styles and Requirements

1. Clusters are individual segments of trimmed and cored cauliflower heads, which measure not less than 20 mm (0.75 in) in the greatest dimension across the top of the unit. A maximum of 10%, by weight, of clusters less than 20 mm (0.75 in) in the greatest dimension across the top of the unit are allowed.
2. Nuggets or small clusters are individual segments of trimmed and cored cauliflower heads, which measure from 6 mm (0.25 in) to less than 20 mm (0.75 in) in the greatest dimension across the top of the unit. A maximum of 20%, by weight, of clusters, 20 mm (0.75 in) or greater, and a maximum of 10%, by weight, of clusters less than 6 mm in the greatest dimension across the top of the unit are allowed.

90.9 FROZEN CORN ON THE COB

Frozen corn-on-the-cob is the product prepared from sound, properly matured, fresh, sweet corn ears by removing husk and silk; by sorting, trimming, and washing to assure a clean and wholesome product. The ears are blanched, then frozen and stored at temperatures necessary for the preservation of the product.

90.9.1 Styles

1. Trimmed. Ears trimmed at both ends to remove tip and stalk ends and or/cut to specific lengths.
2. Natural. Ears trimmed at the stalk end only to remove all or most of the stalk.

90.9.2 Lengths

1. Regular. Ears which are predominantly over $3\frac{1}{2}$ inches in length.
2. Ears which are predominantly $3\frac{1}{2}$ inches or less in length.

90.9.3 Colors of Frozen Corn-on-the-Cob

Golden (or yellow); white.

90.10 FROZEN LEAFY GREENS

Frozen leafy greens are the frozen product prepared from the clean, sound, succulent leaves and stems of any one of the plants listed below by sorting, trimming, washing, blanching, and properly draining. The product is processed by freezing and maintained at temperatures necessary for its preservation. Any functional, optional ingredient(s) permissible under the law may be used to acidify and/or season the product.

90.10.1 Types

- Beet greens
- Collards
- Dandelion greens
- Endive
- Kale
- Mustard greens
- Spinach
- Swiss chard
- Turnip greens
- Any other "market accepted" leafy green

90.10.2 Styles

1. Leaf consists substantially of the leaf, cut or uncut, with or without adjoining portion of the stem.
2. Chopped consists of the leaf with or without adjoining portion of the stem that has been cut into small pieces less than approximately 20 mm (0.78 in) in the longest dimension but not comminuted to a pulp or a puree.
3. Pureed consists of the leaf with or without adjoining portion of the stem that has been comminuted to a pulp or a puree.

90.11 FROZEN OKRA

Frozen okra is the product prepared from the clean, sound, succulent, and edible fresh pods of the okra plant (*Hibiscus esculentus*) of the green variety. The product may or may not be

trimmed, is properly prepared and properly processed, and is then frozen and stored at temperatures necessary for preservation.

90.11.1 Styles

1. Whole okra consists of trimmed or untrimmed whole pods of any length that may possess an edible portion of the cap. The length of a whole pod is determined by measuring from the outermost point of the tip end of the pod to the outermost point of the stem end of the pod, exclusive of any inedible stem portion that may be present.
2. Cut okra is trimmed or untrimmed whole pods, which may possess an edible portion of cap, and which have been cut transversely into pieces of approximate uniform length. The length of a unit of cut okra is determined by measuring the longitudinal axis of the unit.

90.12 FROZEN ONION RINGS, BREADED, RAW, OR COOKED

Frozen breaded onion rings, hereinafter referred to as frozen onion rings, is the product prepared from clean and sound, fresh onion bulbs (*Allium cepa*) from which the root bases, tops, and outer skin have been removed. The onion bulbs are sliced and separated into rings, coated with batter (or breaded), and may or may not be, deep fried in a suitable fat or oil bath. The product is prepared and frozen in accordance with good commercial practice and maintained at temperatures necessary for the proper preservation of the product.

90.12.1 Types

The type of frozen onion rings applies to the method of preparation of the product, and includes:

1. French fried onion rings that have been deep fried in a suitable fat or oil bath prior to freezing.
2. Raw breaded onion rings that have not been oil blanched or cooked prior to freezing.

90.13 FROZEN PEAS

Frozen peas is the food in "package" form, prepared from the succulent seed of the pea plant of the species *Pisum sativum* L. Any suitable variety of pea may be used. It is blanched, drained, and preserved by freezing in such a way that the range of temperature of maximum crystallization is passed quickly. The freezing process should not be regarded as complete until the product temperature has reached $-18^{\circ}C$ ($0^{\circ}F$) or lower at the thermal center, after thermal stabilization. Such food may contain one, or any combination of two or more, of the following safe and suitable optional ingredients:

For more details see:

1. Chapter on Frozen peas: standard and grade.
2. Appendix B.

90.14 PEAS, FIELD, AND BLACK-EYE

Frozen field peas and frozen black-eye peas, hereafter referred to as frozen peas, are the frozen product prepared from clean, sound, fresh, seed of proper maturity of the field pea plant (*Vigna sinensis*), by shelling, sorting, washing, blanching, and properly draining. The product is frozen and maintained at temperatures necessary for preservation. Frozen peas may contain succulent, unshelled pods (snaps) of the field pea plant or small sieve round type succulent pods of the green bean plant as an optional ingredient used as a garnish.

For more details see:

1. Chapter on Frozen peas: standard and grade.
2. Appendix B.

90.15 FROZEN PEPPERS, SWEET

Frozen sweet peppers are the frozen product prepared from fresh, clean, sound, firm pods of the common commercial varieties of sweet peppers, which have been properly prepared, may or may not be blanched, and are then frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

90.15.1 Types

Type I, green; Type II, red; Type III, mixed (green and red).

90.15.2 Styles

1. Whole stemmed: whole unpeeled pepper pods with stem and core removed.
2. Whole unstemmed: whole unpeeled pepper pods with stems trimmed to not more than $\frac{1}{2}$ inch length.
3. Halved: whole stemmed, unpeeled pepper pods which have been cut approximately in half from stem to blossom end.
4. Sliced: whole stemmed, unpeeled pepper pods or pieces of pepper pods which have been cut into strips.
5. Diced: whole stemmed, unpeeled pepper pods or pieces of pepper pods which have been cut into approximate square pieces measuring $\frac{1}{2}$-inch or less.
6. Unit: a whole unpeeled pepper pod or portion of a pepper pod in frozen sweet peppers.

90.16 FROZEN POTATOES, FRENCH FRIED

Frozen French fried potatoes are prepared from mature, sound, white or Irish potatoes (*Solanum tuberosum*). The potatoes are washed, sorted, and trimmed as necessary to assure a clean and wholesome product. The potatoes may or may not be cut into pieces. The potatoes are processed in accordance with good commercial practice which includes deep frying or blanching in a suitable fat or oil and which may include the addition of any

ingredient permissible under the law. The prepared product is frozen and is stored at temperatures necessary for its preservation.

90.16.1 Types

Frozen French fried potatoes are of two types, based principally on intended use, as follows:

1. Retail type. This type is intended for household consumption. It is normally packed in small packages that are labeled or marked for retail sales. It may be otherwise designated for such use.
2. Institutional type. This type is intended for the hotel, restaurant, or other large feeding establishment trade. Primary containers, usually 5 pounds or more, are often not as completely labeled as for retail sales.

90.16.2 Styles

They are grouped under: general, strips, slices, dices, Rissolé.

90.16.2.1 General. The style of frozen French fried potatoes is identified by the general size, shape, or other physical characteristics of the potato units. Styles with cut units may be further identified by substyles as follows:

1. Straight cut refers to smooth cut surfaces.
2. Crinkle cut refers to corrugated cut surfaces.

90.16.2.2 Strips. This style consists of elongated pieces of potato with practically parallel sides and of any cross-sectional shape. This style may be further identified by the approximate dimensions of the cross-section, for example: $\frac{1}{4} \times \frac{1}{4}$ inch, $\frac{3}{8} \times \frac{3}{8}$ inch, $\frac{1}{2} \times \frac{1}{3}$ inch, or $\frac{3}{8} \times \frac{3}{8}$ inch.

Shoestring refers to strip, either straight cut or crinkle cut, with a cross-section predominantly less than that of a square measuring $\frac{3}{8} \times \frac{3}{8}$ inch.

90.16.2.3 Slices. This style consists of pieces of potato with two practically parallel sides, and which otherwise conform generally to the shape of the potato. This style may also contain a normal amount of outside slices.

90.16.2.4 Dices. This style consists of pieces of potato cut into approximate cubes.

90.16.2.5 Rissolé. This style consists of whole or nearly whole potatoes.

Any other individually frozen French fried potato product may be designated as to style by description of the size, shape, or other characteristic that differentiates it from the other styles.

90.16.3 Length Designations

General. The length designations described in this section apply to strip styles only.

Criteria for length designations of a sample unit. Frozen French fried potato strips are designated as to length in accordance with the following criteria. Percent, as used in this section, means the percent, by count, of all strips of potato that are $\frac{1}{2}$-inch in length, or longer.

1. Extra long. Eighty percent or more are 2 inches in length or longer; and 30% or more are 3 inches in length or longer.
2. Long. Seventy percent or more are 2 inches in length, or longer; and 15% or more are 3 inches in length or longer.
3. Medium. Fifty percent or more are 2 inches in length or longer.
4. Short. Less than 50% are 2 inches in length or longer.

90.17 FROZEN POTATO, HASH BROWN

Frozen hash brown potatoes are prepared from mature, sound, white, or Irish potatoes (*Solanum tuberosum*) that are washed, peeled, sorted, and trimmed to assure a clean and wholesome product. The potatoes so prepared are blanched, may or may not be fried and are shredded or diced or chopped and frozen and stored at temperatures necessary for their preservation.

90.17.1 Styles

1. Shredded. Shredded potatoes are cut into thin strips with cross-sectional dimensions from 1 mm × 2 mm to 4 mm × 6 mm and formed into a solid mass before freezing.
2. Diced. Diced potatoes are cut into approximate cube shape units from 6 mm to 15 mm on an edge and loose frozen. They contain not more than 90 g, per sample unit, of units smaller than one-half the volume of the predominant size unit.
3. Chopped. Chopped potatoes are random cut pieces predominantly less than 32 mm in their greatest dimension and loose frozen.

90.18 FROZEN VEGETABLES, MIXED

Frozen mixed vegetables consist of three or more succulent vegetables, properly prepared and properly blanched; may contain vegetables (such as, small pieces of sweet red peppers or sweet green peppers) added as garnish; and are frozen and maintained at temperatures necessary for the preservation of the product.

90.18.1 Kinds and Styles of Basic Vegetables

It is recommended that frozen mixed vegetables, other than small pieces of vegetables added as garnish, consist of the following kinds and styles of vegetables as basic vegetables:

1. Beans, green or wax: cut styles, predominantly of $\frac{1}{2}$ inch to $1\frac{1}{2}$ inch cuts;
2. Beans, lima: any single varietal type;

3. Carrots: diced style, predominantly of $\frac{3}{8}$ inch to $\frac{1}{2}$ inch cubes;
4. Corn sweet: golden (or yellow) in whole kernel style;
5. Peas: early type or sweet type.

90.18.2 Recommended Proportions of Ingredients

It is recommended that frozen mixed vegetables consist of three, four, or five basic vegetables in the following proportions:

1. Three vegetables. A mixture of three basic vegetables in which any one vegetable is not more than 40% by weight of all the frozen mixed vegetables.
2. Four vegetables. A mixture of four basic vegetables in which none of the vegetables is less than 8% by weight nor more than 35% by weight of all the frozen mixed vegetables.
3. Five vegetables. A mixture of five basic vegetables in which none of the vegetables is less than 8% by weight nor more than 30% by weight of all the frozen mixed vegetables.

91

Frozen Avocados

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91.1 Introduction 998
91.1.1 History 998
91.1.2 Biology 999
91.1.3 Growth, Harvesting, and Cultivars 1000
91.1.4 Production Statistics 1002
91.1.5 Nutritional Value 1003
91.2 Frozen Avocado Paste and Guacamole: Processing Operations 1003
91.2.1 Harvesting 1003
91.2.2 Sorting and Grading 1004
91.2.3 Washing 1004
91.2.4 Size Reduction 1005
91.2.5 Avocado Paste 1005
91.2.6 Mixing 1006
91.2.7 Guacamole 1006
91.2.8 Packaging 1006
91.2.9 Freezing 1006
91.2.9.1 Blast Freezer 1007
91.2.9.2 Cryogenic Freezer 1007
91.2.10 Frozen Storage 1007
91.2.11 Quality Standard 1007
91.3 Frozen Avocado Halves: Processing Operations 1008
91.3.1 Peeling 1008
91.3.2 Freezing 1008
91.3.2.1 IQF Belt Freezer 1008
91.3.3 Frozen Storage 1008

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

91.4 Cold Chain 1008
91.5 Recommended International Code of Practice for the Processing and Handling of Quick Frozen Foods of the Codex Alimentarius Commission (FAO/WHO) 1009
91.5.1 Cold Chain Control: Safety and Quality (HACCP and DCP) 1009
91.5.2 Safety 1010
91.5.3 Quality 1010
91.5.4 Transport and Distribution 1010
91.5.5 Retail 1010
References 1010

91.1 INTRODUCTION

In Mexico, as in other producing countries, avocados are eaten fresh in salads made up of different greens and vegetables, or in a typical Mexican sauce called "guacamole." Mexico is the principal producer and exporter of Hass avocados. However, processed avocado (avocado paste, guacamole and halved), is not exported to the same degree. Another avocado byproduct is oil extract that is becoming more popular in some markets and must therefore be taken into consideration for future export.

91.1.1 History

The avocado originated in the mountainous regions of Mexico and Central America and has been consumed for around 10,000 years. This has been confirmed through carbon testing. The West Indian species did not originate in the Caribbean Islands as the name implies, but rather in the lowlands of Central America. The avocado expanded from there to other areas of Central and South Americas and eventually to Spain in 1601, to Jamaica around 1650, to Mauricio in 1780, and to Asia in approximately 1850. Avocados were first noted in Florida in 1833 and in California in 1856 (Samson 1991).

The word avocado (fruit of the *Persea americana* tree) comes from the Nahuatl word ahuacacuáhuitl which means testicle tree. Archeological evidence, fossil remains and signs of human consumption dating back 7000 to 8000 years ago, and found in Tehuacan, Puebla, indicate that this fruit has been present since 10,000 years ago (León 1987). It was probably a very popular item in the diet of pre-Columbian cultures due to its nutritional and medicinal values (ASERCA 2002). The most ancient examples were found in Peru dated 3500 to 4000 years ago (León 1987).

Commercial exploitation of avocados in California and Florida began in 1932 and later expanded to Chile, Brazil, South Africa, and most recently, to Israel. That is to say, avocados are now grown in tropical and subtropical areas where ecological conditions suitable for the optimal development of this fruit exist.

Smith and others (1992) reported that the original "Fuerte" variety avocado tree first gave fruit in Atlixco, Puebla, Mexico in 1908 and died at the end of the 1940s. These same authors indicated that in 1920 Rudolph Hass discovered the Hass variety in La Habra Heights, California. He patented it in 1935. This variety still yields excellent quality fruit to this day. The Hass variety is grown and commercialized with great success in Mexico.

91.1.2 Biology

The botanical name of the avocado is *Persea americana* and belongs to the *Lauraceae* family. Other known members of this family are the laurel and cinnamon trees. The English name avocado is derived from the Spanish word abogado, an adaptation of the Aztec word ahuacatl. This word in Nahuatl became avocat in French and advokaat in Dutch. The name palta is still used in Peru, Ecuador and Chile (Samson 1991).

Three ecological races are recognized (subspecies or botanical varieties): the Mexican, the Guatemalan, and the West Indian which are considered to be subtropical, semitropical, and tropical, respectively. The physiological differences among the three races are commercially important and related to the time to maturity, fruit size, peel texture, flavor, oil content, disease tolerance, storage characteristics, climate, and cold tolerance of the tree. Mexican cultivars are well adapted to the cool climates of the tropics and subtropics and are the most cold tolerant of the three types. The Mexican cultivars produce fruit with thick peel or rind which is usually smooth and glossy and that mature earlier than, or simultaneously with, the West Indian cultivars (Salunkhe and Desai 1986; Samson 1991).

The absence of a sterility barrier between population types and the mechanism of floral biology favor the formation of hybrid populations. Many of the commercial crops that are vegetatively propagated come from interracial crosses.

The avocado tree has a very shallow root system, leaves that are alternately arranged, and bunches of buds. The inflorescences are found in the thousands, each with hundreds of flowers: these are green and are 1 cm wide and deep, have verticils with three stamens, and an ovary. It is therefore a complete flower and opens twice, closing in the interim: the first time it acts as a female; the second, as a male (Samson 1991). *Persea americana* produce thousand of flowers per plant: the panicles open for long periods, from weeks to months. Nevertheless, the number of flowers which are fertilized and produce fruit is low (León 1987).

The characteristics of the fruit vary considerably, depending on the type and race and cultivar. Pear shaped fruit is the most dominant, although there are also spherical and elongated oval shaped fruit. They are generally asymmetrical, with a thicker side which is more fibrous or has vascular bundles. The external color varies from yellowish green to purple or almost black. The surface can be smooth and shiny, or rugged pebbly brown-black. Hass avocados have a thick, rough, bright green peel, varying from dark green to almost black, depending on its degree of ripeness (Fig. 91.1). Its pulp is pale green and creamy textured and has a characteristically delicious taste.

The external tissue, epidermis and hypodermis of the fruit, separates easily from the meaty mesocarp which is rich in oils and makes up the edible part. The epidermis, consisting of strong walled isodiametric cells, has numerous stomas and is covered by a waxy layer. There are also lenticels, small lighter colored protuberances that frequently open, forming breaks on the peel of the fruit that are later covered with a corklike substance. The hypodermis is composed of parenchyma cells, full of chloroplasts and resins (Biale and Young 1971). There is a discontinuous layer of esclerenchyma underneath composed of groups of very thick walled cells which give the rind its characteristic grainy texture that in certain types is very easily pierced. This is the reason why the peel is separated from the rest of the fruit and what determines the thickness of the former. The mesocarp is rich in oil that can constitute up to 30% of the total weight. This oil is very nutritious, easy to digest, and increases as the fruit matures, completely filling many of the cells in the mesocarp while also appearing in other spherical

Figure 91.1 *Hass avocado* (*Persea americana*).

bodies (Fig. 91.2). The endocarp consists of several layers of thinned walled cells called parenchyma which often adhere to the seed testa (Biale and Young 1971; León 1987).

The ovoid shaped seed takes up a great part of the fruit: it is made up of two meaty cotyledons and a small embryo; it does not contain endosperm. The testa is made up of five external layers of sclerenchyma and various layers of parenchyma; the layer closest to the sclerenchyma layer is filled with tannin which gives it its characteristic dark color. The cotyledons are mainly made up of parenchyma which contain starch and tannins (León 1987).

91.1.3 Growth, Harvesting, and Cultivars

The fruit of the avocado tends to grow continually on the tree and matures after it is harvested. This characteristic has been used to store the fruit on the tree for a few months.

It is climacteric: its pattern of respiration is characterized by the so-called "climacteric increase." Ripening is triggered by endogenous ethylene and it has been established that a

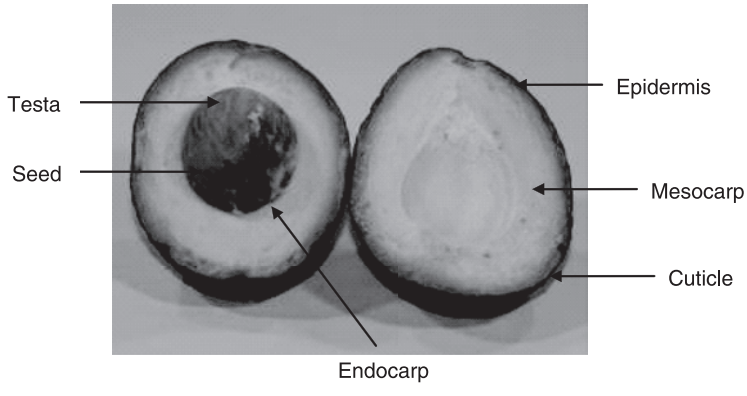

Figure 91.2 *Cross-section of an avocado.*

concentration of 10 ppm of ethylene at 15–17°C is enough to begin ripening in "Fuerte" avocados (Yahia and Báez 1992).

There are physiological differences in the three types (Mexican, Guatemalan, and West Indian) which include size and shape of the fruit, taste, oil content, disease tolerance, climate adaptations, and storage requirements. Mexican cultivars are adapted to cool climates and have the largest highest oil content of the three. The Mexican cultivars are "Gottifried" and "Pernod"; the "Fuerte" avocado is a hybrid of the Mexican and Guatemalan. The most important variety cultivated in Mexico is "Hass," and of lesser importance are the "Fuerte," "Booth 7," "Booth 8," "Azteca," "Zutano," "Bacon," "Atlixco," "Duke," "Perfecto," "Sinaloa," "San Sebastián," and "Colin V33" varieties (Yahia and Báez 1992; ASERCA 1999).

The "Fuerte," which originated in Atlixco, Puebla, is grown in other producing states. Its main characteristic is its resistance to cold, but sensitivity to heat. In Coatepec de Harinas, Mexico, the new V33 selection of the Fundación Salvador Sánchez Colín CICTAMEX is a variety of low stature which permits the efficient control of diseases and production through a greater ease in pruning and similar labor on each tree (ASERCA 1999).

The state of Michoacan produces 87% of the avocado production in Mexico and is the principal exporter to the United States (SIAP 2006). Creole avocados were grown in Michoacan until 1941 when the Fuerte variety was successfully introduced, triggering it to become the most productive avocado region in the world. Nevertheless, years later, the Fuerte variety was displaced by the Hass cultivar, originating in California and Florida. This variety had higher competitive advantages. The Hass avocado was preferred by producers because of its:

- Sustained productivity;
- Low, crop alternation;
- Compact growth pattern;
- Tolerance to handling and transport;
- The excellent quality of its pulp.

Once its quality was proven, the orchards were quickly grafted again with the Hass variety and new plantations are now established with this variety (ASERCA 1999).

Avocados are found all year round because there are different varieties, predominantly of the Hass cultivar. The harvest seasons are in two stages: the high season occurring from October to January, and the low season from February to September. There are four flowerings so it is recommended that the harvest be done in the following manner: (1) the fruit of the crazy flowers in August and September, (2) the fruit of the advantaged which has a head start, is harvested from October to December, (3) with the normal flowers, the fruit is harvested from January to February, (4) with the "march flower," the fruit is harvested beginning in March (ASERCA 1999).

With regards to avocado exports, the Unites States Department of Agriculture (USDA) has established a strict and costly import program which began on February 5 1997; on this date the Federal Register published the authorization for Mexican avocados to be exported to 19 east coast states in the United States.

The packinghouses are supervised during the entire export season to the United States, under the verification stage of the import program established by the USDA. The avocado

is transported from plantation to the packinghouse with shipment being covered with mesh and lots are not mixed. Once a lot has been packed, all the equipment used for processing in the packinghouse is cleaned before beginning with another lot.

The Michoacan Association of Producers, Packers and Exporters, A.C. in charge of the export program to the United States, is organized and has strict rules which has successfully permitted it to place excellent quality avocados in the North American market with confidence.

The harvest is done through a planned program to order the fruit picking for export. This is a complex process where various factors such as the economic needs, the climate (the orchards are located in hot areas, as well as where frost is a risk), the ripeness of the fruit and quality specifications, among others are considered (ASERCA 2002).

91.1.4 Production Statistics

Avocados are harvested all year round and grown in 28 states of Mexico. Nevertheless, in 2003, 95.5% of the national production was concentrated in five states in decreasing order, Michoacan, 88.44%; Morelos, 2.65%; Nayarit, 1.71%; Puebla, 1.40%; and the state of Mexico, 1.30% (Table 91.1, INEGI 2005). Michoacan has advanced technology for the growth and postharvest handling of Hass avocado and is therefore the state that exports this fruit to the United States of America, Japan, and Europe.

At the world level, Mexico is the principal avocado producer, after the United States, Indonesia, Brazil, Colombia, and Chile. These six countries produced 61.74% of the world production in 2004 (Table 91.2; FAOSTAT 2005).

The first export season of Mexican avocado to the United States was 1997/1998 comprised by November 5 1997 to February 22 1998 when 348 shipments for a total of 6031.7 tons. Avocado exports to this country have increased from this date. France was previously the main market for fresh and processed Mexican avocado (ASERCA 2002). The principal exporting countries in 2004 were Mexico, Chile, Israel, Spain, and South Africa. The main importers during the same year were the United States, France, the Netherlands, the United Kingdom, and Japan (FAOSTAT 2005).

Avocado consumption is concentrated in the major producing areas; for example, Mexico, the United States of America, Brazil, and so on. Mexican per capita consumption is about 7–8 kg per person, compared with U.S. consumption of less than 1 kg. Mexico is the main consumer at the world level (USDA 1997).

TABLE 91.1 Principal Avocado Producing States in México, 2003.

State	Area Harvested (1000 Ha)	Production (1000 Metric ton)	Participation (%)
National	103.3	905	100
Michoacan	80.9	800. 4	88.44
Morelos	2.5	24.0	2.65
Nayarit	2.2	15.5	1.71
Puebla	2.3	12.6	1.40
Mexico State	1.6	11.7	1.30
The others	13.8	40.8	4.50

Source: Adapted from INEGI 2005.

TABLE 91.2 Principal Avocado Producing Countries, 2004.

Country	Area Harvested (1000 Ha)	Production (1000 Metric ton)	Participation (%)
World	417	3078	100
Mexico	102.5	1040.4	33.80
USA	27	200	6.50
Indonesia	70	177.2	5.75
Brazil	13	175	5.69
Colombia	15	158	5.13
Chile	24.5	150	4.87
The others	168	1177.4	38.26

Source: Adapted from FAOSTAT 2005.

TABLE 91.3 Avocado Composition Based on 100 g of Edible Material.

Water (g)	Kilocalories	Protein (g)	Fat (g)	Total Carbohydrates (g)	Fiber
73.2	160	2.0	14.7	8.5	6.7

Source: Adapted by Cano and others (2005).

91.1.5 Nutritional Value

The composition of an avocado is affected by the variety, ripeness, climatic growing conditions, light, temperature, soil, fertilization, and irrigation. Its pulp is 73.2% water, has a high oil content (14.7%), is rich in proteins and contains vitamin A and C, has a relatively low sugar content, and contains minerals such as calcium, magnesium, potassium, and phosphorus (Table 91.3; Cano and others 2005).

91.2 FROZEN AVOCADO PASTE AND GUACAMOLE: PROCESSING OPERATIONS

In Mexico avocados are mainly eaten fresh in salads and in a sauce called guacamole. The term *guacamole* comes from the Nauatl *ahuaca-mulli* which means avocado paste. In Mexico, the oldest and most basic form of guacamole is avocado flesh, some coriander leaves, onion, garlic and chopped jalapeño peppers ground together in a "molcajete" (stone mortar and pestle). It was always served as a sauce for meat or simply rolled inside a "tortilla." Guacamole, the paste, and frozen avocado halves are exported to the United States and Europe. The main industrial avocado processing plants are located in Michoacan. In 2001, Mexico exported 42,980 tons of guacamole (ASERCA 2002). Other producing countries besides Mexico such as the United States and Spain make the guacamole salsa taking into consideration the Mexican style of the sauce.

91.2.1 Harvesting

Avocados are manually harvested; the fruit in the lowest parts of the tree are cut with scissors and placed in harvesting bags. Hooks with knives are also used to separate the fruit from the higher tree branches. The harvesting bags are emptied into boxes in the field where the fruit is selected for fresh export and for processing.

91.2.2 Sorting and Grading

Sorting is the separation of fruit into categories on the basis of measurable physical properties. Like washing, sorting should be done as early as possible to ensure a uniform product for subsequent processing. The four main physical properties used to sort avocados are size, shape, weight, and color. Grading means the assessment of a number of attributes to obtain an indication of overall quality of a fruit or vegetable. Sorting (that is, the separation on the basis of one characteristic) may therefore be used as part of a grading operation (Fellows 1997). Trained operators grade avocados (Fig. 91.3).

91.2.3 Washing

The avocados are transported on a moving belt to an immersion tank where they are washed and subsequently brushed. Thorough washing removes dirt, mold, and other contaminants. In order to improve the washing efficiency, detergent or chlorine is also added to decrease the microbial load.

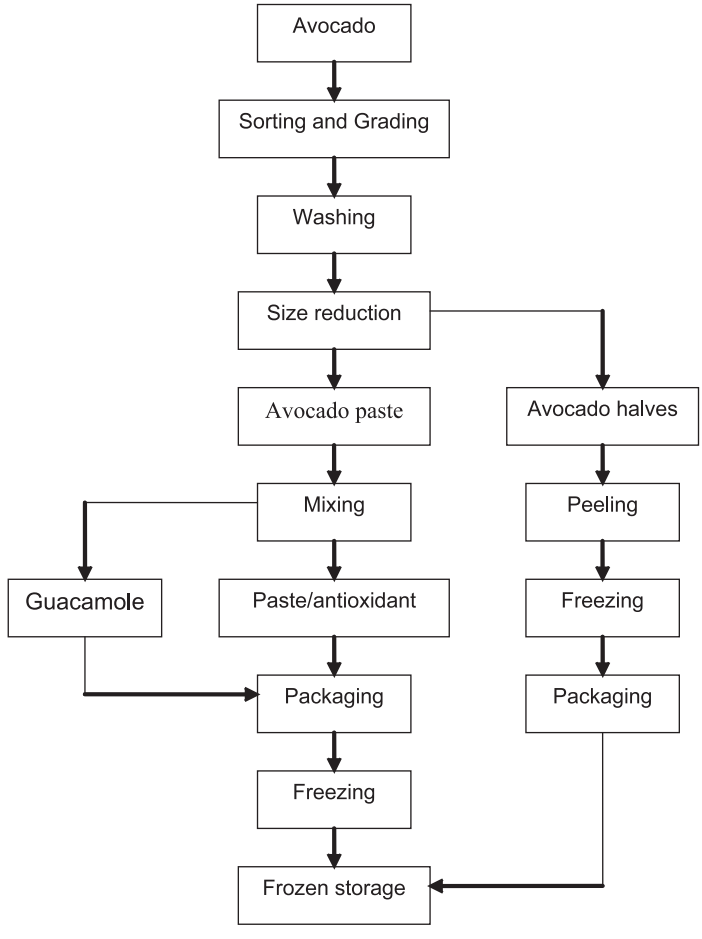

Figure 91.3 *Flow chart for avocado paste and halves, and guacamole.*

91.2.4 Size Reduction

Size reduction is the unit operation in which the average size of solid pieces of food is reduced through grinding, compression or impact forces. This operation has benefits in food processing such as: (1) there is an increase in the surface-area-to-volume ratio of the food which increases the drying, heating, or cooling rate and improves the efficiency and extraction rate of soluble components (for example juice extraction from cut fruit); (2) a similar range of particle size allows ingredients to be mixed more thoroughly (Fellows 1997). Avocado paste and additives are examples of such mixtures.

Different methods of size reduction are classified according to the particle size range produced:

1. Chopping, cutting, slicing, and dicing;
2. Milling to powders or pastes of increasing fineness;
3. Emulsification and homogenization.

Most fruits and vegetables fall into the general category of fibrous foods, have a firmer texture, and are cut at room or chilled temperatures. Avocados are cut in half with slicing equipment that consists of rotating or reciprocating blades that cut the fruit. The seeds are manually removed from the avocado halves by operators (Fig. 91.4).

91.2.5 Avocado Paste

Avocado halves are sent to a hammer or roller mill where the pulp is separated from the peel and the particle sizes are homogenized to make the paste.

In the roller mill, two rollers revolve towards each other and pull the avocado halves through the “nip” (the space between the rollers). The main force is compression, but if the rollers are rotated at different speeds, or if the rollers are fluted, there is an additional shearing force exerted on the fruit (Brennan and others 1980; Fellows 1997).

In the hammer mill, avocados are disintegrated mainly by impact as the hammers drive the avocado against the breaker plate. A horizontal cylindrical chamber is lined with a

Figure 91.4 *The selection of avocado halves by operators.*

toughened steel breaker plate and a high-speed rotor inside the chamber is fitted with hammers along its length.

91.2.6 Mixing

Mixing or blending is a unit operation in which a uniform mixture is obtained from two or more components by the dispersion of one into the other. This operation has very wide applications in many food industries where it is used to combine ingredients to achieve different functional properties or sensory characteristics. Antioxidants such as ascorbic acid or citric acid are added to the avocado paste that is then conveyed to the packing line. Antioxidants must be added to the avocado paste to inactivate the polyphenoloxidase.

91.2.7 Guacamole

Guacamole is prepared in the mixer with avocado paste, onion, garlic, coriander, jalapeño peppers, and salt. The product is conveyed to packaging line.

91.2.8 Packaging

Guacamole or avocado paste packaging is done in semi-rigid containers. Plastic cans are made from laminated polyvinylidene chloride-coated polypropylene-polyethylene film (Fig. 91.5; Fellows 1997) which is sealed with easy-open aluminum ends. The excess air is mechanically removed from the containers which are sealed under vacuum.

91.2.9 Freezing

Freezing is the unit operation where the temperature of a food is reduced to below its freezing point, and a proportion of the water is transformed into ice crystals. The immobilization of water into ice and the resulting concentration of dissolved solutes in unfrozen water lowers the water activity of the food. Preservation is achieved by a combination of the low temperatures, reduced water activity and in vegetables, pretreatment through blanching (Fellows 1997).

Figure 91.5 *Plastic packages for avocado paste and guacamole.*

Freezing is an effective means of preservation that maintains the quality of fruits or vegetables similar to the fresh product. Though this method is not cheap, the product retains more nutrients. Fruits and vegetables retain their natural color, flavor, and texture better when they are frozen. Although this unit operation delays the enzymatic activity, it does not stop it. In the case of avocado paste, it cannot be blanched due to its oil content. The color and flavor of avocado paste undergo damage when the paste was heated at 73–85°C for 4.6–10 min (Ortiz and others 2003). The addition of antioxidants such as ascorbic acid is an alternative method (Ramírez and others 1992).

The freezing process is dependent on freezing rate, heat transfer coefficient, and amount of heat removed from the food product. The freezing time depends on freezing rate, amount of heat removed, packaging and freezing methods used, initial and final temperature desired, thickness of the product, and food ingredients (Martinez-Romero and other 2004).

91.2.9.1 Blast Freezer. Avocado products are frozen in the food industry using different methods. Avocado paste and guacamole are frozen in a blast freezer. Air is recirculated over the packaged avocado paste at between −30°C and −40°C at a velocity of 1.5–6.0 m/s. In batch equipment, the product is stacked in trays in rooms or cabinets. Continuous equipment consists of a conveyor belt that carries the food through an insulated tunnel.

91.2.9.2 Cryogenic Freezer. A cryogenic freezer is also used to freeze avocado paste and guacamole. Freezers of this type are characterized by a change of state in the refrigerant (or cryogen) as heat is absorbed from the freezing food. The cryogen is in direct contact with the food and rapidly removes energy from the food to provide its latent heat from vaporization or sublimation, to produce high heat transfer coefficients and rapid freezing. The two most common refrigerants are liquid nitrogen and solid or liquid carbon dioxide. In a liquid-nitrogen freezer, packaged avocado paste travels on a perforated belt through a tunnel, where it is cooled by gas nitrogen and is then frozen by liquid-nitrogen sprays. The temperature is allowed to reach the required storage temperature (between −18°C and −20°C) before the product is removed from the freezer (Fellow 1997).

91.2.10 Frozen Storage

Avocado products will maintain high quality for 18–20 months at −18°C to −20°C. However, during frozen storage, the number of ice crystals will be reduced, while their size increases. These changes are affected by fluctuations in storage temperature, which in turn can cause migration of water vapor from product to surface of the container. The increase of ice crystals during prolonged frozen storage induces drip loss. Also, physical and chemical changes can occur. Main physical changes in fruits and vegetables products during frozen storage are due to recrystallization and sublimation phenomena related to the stabilization crystals inside the product and on the outside surface (Reid 1991; Martínez-Romero and other 2004). The recrystallization rate decreases at low temperatures, with no ice crystal growth at temperatures below −20°C (Reid 1991).

91.2.11 Quality Standard

There is only one quality norm for fresh Hass avocado (NMX-FF-016-SCFI-1995). This norm takes into account the minimum quality requirements that the fruit (a minimum of

21.5% dry material) must meet, three grades of quality (supreme, class I and class II), tolerances, testing methods, marking and labeling, packaging, and storage. This Mexican standard is in concordance with the international avocado norm of the Codex Alimentarius Commission (CODEX STAN-197-1995). International specifications such as: pH, oil content, salt content, viscosity, microbiological analysis, and product temperature are taken into account with processed avocado products.

91.3 FROZEN AVOCADO HALVES: PROCESSING OPERATIONS

The sorting, grading, washing, and size reduction operations are the same as used to make avocado paste and guacamole.

91.3.1 Peeling

Operators remove the peel, or rinds, from the avocado halves which then frozen.

91.3.2 Freezing

The avocado halves can be frozen using an IQF belt freezer, blast freezer or cryogenic freezer.

91.3.2.1 IQF Belt Freezer. The avocado halves are inspected, and frozen on an IQF belt freezer: the avocado halves are fluidized and quickly crust frozen in the first zone. Freezing of the product is completed on a second belt at $-18^{\circ}C$. Frozen avocado halves are packed into bags in boxes (polyethylene-in-polypropylene/carton).

91.3.3 Frozen Storage

Frozen avocado halves are stored in a freezer at a range of $-25^{\circ}C$ and $-30^{\circ}C$. The product must have a temperature of $-18^{\circ}C$. Frozen avocado products have a shelf life of 18–20 months and 1–2 days when refrigerated after removal from storage.

91.4 COLD CHAIN

It is important to consider the cold chain in the production-distribution of frozen avocado products to maintain their quality and safety until they reach the consumer.

The cold chain is composed of all the processing phases from rapid freezing until the product reaches its final consumer. This chain is established based on the fact that the frozen product must maintain a temperature equal or lower than $-18^{\circ}C$ throughout its entire commercial distribution process (Rodríguez and others 2002). Each link in the commercial cold chain follows the guides below:

- Freezing installation for freezing the product at $-18^{\circ}C$;
- Freezer storage at a range between $-25^{\circ}C$ and $-30^{\circ}C$, until the frozen product is shipped;

- Freezer vehicles used for transport to the distribution deposits at $-25^{\circ}C$;
- Distribution deposits' temperature of $-20^{\circ}C$;
- Distributor vehicles to the selling center at $-20^{\circ}C$;
- Selling centers' temperatures between $-18^{\circ}C$ and $-20^{\circ}C$.

The transport and household cold must also be considered as links in the cold chain. That is these noncommercial links should keep the temperature of the frozen product below or equal to $-18^{\circ}C$. Otherwise, the texture of product can be damaged by recrystallization.

91.5 RECOMMENDED INTERNATIONAL CODE OF PRACTICE FOR THE PROCESSING AND HANDLING OF QUICK FROZEN FOODS OF THE CODEX ALIMENTARIUS COMMISSION (FAO/WHO)

The countries that are members of the Codex Alimentarius Commission are revising the recommended international code for the processing and handling of quick frozen foods. The United States, as the host country of the Committee on Processed Fruits and Vegetables and the Committee on Food Hygiene (Codex Alimentarius Commission 2005), is coordinating the work.

The code considers the application of a program to control the dangers or defects identified throughout the cold chain. The concept of defect correction points, (DCP), was introduced and makes a clear distinction between the aspects related to quality and those involving safety (critical control points). It also includes temperature control as part of the management of the cold chain (freezing chambers, quick freezer, transport, retail sales) (Codex Alimentarius Commission 2001).

Defect: a defect is defined as the condition of a product that does not meet the essential dispositions of quality, composition and/or labeling which correspond to the Codex standard for products.

Defect correction point (DCP): the defect correction point is the stage where it is possible to apply control and avoid, eliminate or reduce a defect to an acceptable level, that is, eliminates the risk of an incorrect description.

The Practices Code incorporates the focus of Hazard Analysis and Critical Control Point System (HACCP); and establishes the technological directives and essential hygiene requirements for the processing of harmless quick frozen food products for human consumption which satisfy the corresponding norms of the Codex about these products.

91.5.1 Cold Chain Control: Safety and Quality (HACCP and DCP)

In the case of fruits and vegetables, these are some stages that can present defect correction points.

Some varieties of fruits and vegetables are more suitable for freezing than others (since they suffer less loss through exudation, or have a more adequate texture after thawing). To test for the correct varieties being received can constitute a DCP.

Blanching is more frequently used in the production of frozen vegetables, which reduces the microbiological charge, though its main objective is the deactivation of the enzymes that cause quality defects (taste, color) during freezer storage. Blanching constitutes a DCP and is considered to be a critical control point (CCP) in only some cases.

When it is necessary to store fruits or vegetables (fresh or intermediate products) before further processing, the storage condition, particularly temperature, must be appropriate for the given product. This stage could be a CCP or a DCP.

91.5.2 Safety

When large lots of vegetables or fruits are frozen it is necessary to leave space or canals that permit air circulation between the product in bulk or the containers. If such areas are not available, freezing will be slow.

It is important that the heat center of the product be cooled as quickly as possible to avoid the development of pathogenic microorganisms or the production of microbiological toxins. The freezing time can constitute a CCP.

91.5.3 Quality

The temperature must be quickly reduced in order to retard spoilage in quick frozen fruits and vegetables. Generally speaking, the faster the freezing takes place, the smaller the size of the crystals formed and the quality is not affected. The freezing time can constitute a DCP.

91.5.4 Transport and Distribution

Quick frozen fruits and vegetables are transported in vehicles that maintain the product at a temperature of $-18^{\circ}C$ or less. The temperature of the product while being transported and distributed constitutes a DCP.

91.5.5 Retail

Quick frozen fruits and vegetables must be sold in freezer units that maintain the product temperature at $-18^{\circ}C$. The freezer unit temperature constitutes a DCP.

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92

Frozen French Fried Potatoes and Quality Assurance

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92.1 Introduction 1014
92.2 Products Covered 1014
92.3 Production 1015
92.3.1 Areas of Production 1015
92.3.2 Varieties 1015
92.3.3 Receiving 1015
92.3.4 Determining the Quality and Condition of Raw Potatoes for Frying Purposes 1016
92.4 Manufacture 1016
92.4.1 Washing 1016
92.4.2 Peeling 1016
92.4.3 Trimming 1017
92.4.4 Slicing 1017
92.4.5 Sizing 1017
92.4.6 By-products 1017
92.4.7 Desugaring 1018
92.4.8 Blanching 1018

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

92.4.9 Frying 1018
92.4.10 Fat or Oil 1018
92.4.11 Time and Temperature 1019
92.4.12 Packaging 1019
92.5 Inspection During Packing Operations 1020
92.6 Inspecting the Product 1020
92.6.1 Sample Unit Size 1020
92.6.2 Initial Fry Color, Types, Styles, and Length Designations 1020
92.6.2.1 Fry Color Definitions 1020
92.6.2.2 Fry Color Designations 1021
92.6.2.3 Refry Color Definitions 1021
92.6.2.4 Types 1021
92.6.2.5 Styles 1022
92.6.2.6 Length Designations 1022
92.6.3 Preparation of Sample 1023
92.7 Quality Evaluation 1023
92.7.1 Grade Factors Which are not Scored 1023
92.7.1.1 Flavor 1023
92.7.2 Color Designation of a Sample Unit 1024
92.7.3 Uniformity of Size and Symmetry 1024
92.7.4 Defects 1025
92.7.4.1 Considerations 1025
92.7.4.2 Defect Tables in the Standards 1025
92.7.5 Assigning the Score for Defects 1025
92.7.5.1 Procedure 1025
92.7.5.2 Texture 1027

92.1 INTRODUCTION

This chapter describes the general process of manufacturing frozen French fried potatoes and some aspects of quality assurance of the products. The information has been derived from grading and inspection documents issued by the U.S. Department of Agriculture.

92.2 PRODUCTS COVERED

The principal products covered are the traditional “French fries” potatoes cut into strips, partially deep fried, and frozen. The standard also may be applied to any potato product, regardless of shape or composition which is similarly processed and frozen. This includes products fabricated primarily from mashed, crushed, cut, or shredded potatoes and which are preformed into units prior to frying and freezing. Because of the difficulty of keeping oils in suitable condition, deep frying has not been popular with home cooks. With the discovery and development of frozen French fries, home consumption has increased rapidly. Institutional use also is increasing yearly. Some believe that yearly production now far exceeds any other frozen vegetable.

92.3 PRODUCTION

92.3.1 Areas of Production

The white potato is the world's most important vegetable crop. It is grown to some extent in all agricultural areas in the United States. Certain types of potatoes particularly those of low solids content are not always suitable for manufacturing. Therefore, extensive production of frozen french fried potatoes is limited principally to those areas where the raw product is most suitable. They are in general the Idaho, Eastern Oregon, and Washington areas, the San Joaquin Valley of California, the state of Maine, with some sizeable production in New Jersey, Eastern Pennsylvania, Michigan, and the Red River Valley of Minnesota.

Because of varietal differences and growing conditions potatoes from these widely separate areas have their own characteristics particularly with respect to flavor and mealiness. Very mealy French fries are produced principally from the Russett varieties in the Pacific Northwest. In other sections of the country the solids of the raw product are generally lower and the finished French fries have a slightly different flavor and are less mealy than those from the Northwest region. These regional differences have given rise to claims of superiority of the product based principally on the degree of mealiness. This is partially a matter of personal preference. Good quality French fries are produced in all the leading producing areas.

92.3.2 Varieties

There are several dozen recognized market varieties of potatoes grown in the United States and more are being developed each year. The Irish Cobbler is probably the most widely grown and the Katahdin is grown in the greatest volume. Among the more popular varieties are the Russet Burbank (Idaho), Cobbler, Katahdin, White Rose, Green Mountain, Bliss, Triumph (red), Russett Rural, Kennebec, Norgold, and Pontiac. Various varieties of potatoes have their own cooking qualities. Some are more popular for one quality than for another, that is, bakers, boilers, and fryers. The characteristics of the various varieties are not distinct and they are not always the same in all growing areas and all seasonal conditions. Therefore, no one variety or varieties is used entirely for the production of frozen French fried potatoes.

92.3.3 Receiving

Frozen French fries are usually produced fairly close to the source of supply but occasionally the raw product may be drawn from any of the principal potato producing areas of the country. At time of harvest most late varieties of potatoes have a total sugar content of less than 1% of total solids. Such potatoes are usually suitable for manufacturing into frozen French fries. After the potatoes are stored for a period of time at 40° or less the starch content partially changes to sugar and the potatoes if used immediately out of storage maybe unsatisfactory because of the high sugar content. Sugar in excess of 2–3% (based on dry potato weight) may render the potato practically worthless for deep frying. Such potatoes subjected to high temperatures develop black or brown areas, spots, or streaks, due to carmalizing and burning of the sugar.

They also may have a burnt or sweet taste. Most potatoes can be "conditioned" by storing for a period of time, at least 2 weeks, at near 60 or 70° temperature. In "conditioning" the potato starts to respire, a process which uses the sugar and converts a portion of it back to starch. If the potatoes have been subjected to excessive cold storage, that is down to 32–34° or lower, trouble in conditioning may be encountered, such as tissue breakdown leading to rotting.

92.3.4 Determining the Quality and Condition of Raw Potatoes for Frying Purposes

Processors try to evaluate and classify the quality of the raw product prior to purchase or processing. Two of the most important characteristics that indicate quality are specific gravity, closely associated with moisture content, and the degree to which starch has been converted to sugar. These will affect the texture and the color of the product. The size and shape of the potatoes is also important because of the cost of operations, the yield, the length of the units, and the number of slivers and irregular-shaped pieces. The presence of off-odors and off-flavors such as those caused by some insecticides is at times very serious.

No entirely satisfactory method seems to have been developed to predetermine the cooking quality of potatoes. Specific gravity tests, which to some extent indicate the degree of mealiness, are sometimes made. Picric acid color tests may also be made. These indicate to some extent the relative amount of sugars present. The objectionable flavor of benzine hexachloride – an insecticide – can be detected by boiling and mashing a sample of the potatoes.

Probably the most satisfactory method of determining the quality of the raw product is to subject a representative sample of the lot to a cooking test similar to the process which will be used in manufacture. The USDA in cooperation with the state of Maine and certain potato processors have developed a series of color photographs which show various degrees of darkening after a standard fry. Comparison of actual samples of cooked potato to the photographs provides a fairly accurate means of evaluating the quality of a load of potatoes for the purpose of making French fries. Some large users base their raw potato contracts on the fry colors shown in the USDA Color Standards for Frozen French Fried Potatoes.

92.4 MANUFACTURE

Each processor of frozen French fried potatoes has his own particular methods of manufacture. However, there are a number of things common to all processors. The following outline describes the principal steps in manufacture. These steps may vary with different manufacturers. The principles given here are basic.

After receiving potatoes or having withdrawn them from storage bins or "conditioning" cellars frying and/or suitable chemical tests are made from representative samples of the lot to determine whether potatoes are in condition to be processed.

92.4.1 Washing

If potatoes are in condition suitable for processing they are washed and may be run through hot water to remove some of the dirt and to loosen the peel. The potatoes may then be sized prior to peeling. Some plants flume the potatoes from place of storage to the peelers thus accomplishing the preliminary washing in this manner.

92.4.2 Peeling

After washing off excessive dirt the potatoes are dropped into peeling machines. These may be steam, lye, abrasive, or roller type peelers. The steam and lye peelers give a quick cook which loosens the skin or peel but does not penetrate deeply into the potatoes.

The peelings are then removed by passing the potatoes through rubber rollers and water sprays. In abrasive, roller type peelers the skin or peel is removed without the addition of heat.

92.4.3 Trimming

The potatoes after leaving the peeling machines are trimmed on wide moving belts. In the better plants these belts are arranged in sections so that each potato is picked up by an operator, examined for defects, trimmed if necessary, and tossed over a barrier onto another section of the belt. This procedure is much more satisfactory than trying to stir the potatoes on a single belt because many potatoes may miss any examination at all on the single belt. At this time the potatoes may also be sorted for size; the larger ones going to institutional lines; the smaller ones into the retail and by-product lines.

In some plants electric eye sorters are installed after the slicing operation to eliminate blemished units, thus cutting down on the amount of hand sorting and trimming of the whole potatoes.

92.4.4 Slicing

After the potatoes are trimmed and sorted to size they go to the slicing machines. These slicers usually consist of two set of knives either rotary or fixed. One set of knives slices the potato to the desired thickness. The potato slices are then passed through another set of knives, which cut the slices to strips if desired. The size of the strips depends on the wishes of the management. It may vary from one-quarter by one-quarter inch to one half by one half inch in cross-section. The usual size for retail sales is $\frac{3}{8}$ by $\frac{3}{8}$ inch. Poor slicing may be caused by small or irregular-shaped potatoes, by poor machinery, or good machinery not properly used or adjusted. The knives may be straight or corrugated.

92.4.5 Sizing

In the process of cutting potatoes into strips, there is always a certain amount of slivers and otherwise irregular shaped pieces. A certain number of these more or less irregular shaped pieces are expected in this product and are allowed for in the tolerances contained in the grade standards. It is usually necessary, however, to pass the cut potatoes over some type of shaker screen to remove a portion of the small pieces and slivers. The amount of chip material removed depends to some extent on the wishes of the purchaser. Processors do not like to remove any more than they have to because of the loss in yield.

92.4.6 By-products

The excessive loss of potato material because of the peeling, trimming and screening operations causes processors to consider by-products to utilize this material. Often this material is wasted; however, a large number of products, such as patties, puffs, shreds, diced, and mashed have been developed to utilize this material. Dehydrated flakes is also an important use. Where satisfactory use is made of screenings and sound throw-outs, there is less tendency to keep this material in the frozen French fry pack.

92.4.7 Desugaring

Sugar in excessive amounts or irregular quantities of sugar between units may cause French fried potatoes to have dark or irregular color, poor texture, and/or unpleasant taste. Proper harvesting, good storage, and conditioning after storage helps in the control of the sugars. However, conditioning and storing potatoes is an expensive process and is avoided whenever possible. Reasonably satisfactory methods of rapid equalization of sugar content has been developed. The methods used vary between manufacturers. However, the basic principle is to run the sliced potatoes through a water bath leaching out a portion of the surface sugar and then replacing the sugar to the desired level by blanching in a sugar solution (partially cooking the product) so that upon frying the color between units will be uniform. This method, based on a patented process, evens the surface sugar content between units. The sugar content of the whole slice is not greatly affected.

92.4.8 Blanching

The sliced potatoes are usually run through a hot water blanch which partially cooks the product. This may or may not be a part of a desugaring process referred to previously. After blanching the product may pass beneath heating units, under forced draft, which tends to remove most of the excessive moisture before entering the fryer.

92.4.9 Frying

Frying of the potatoes is usually a continuous process. The potatoes enter the hot oil on or under a draper-chain type belt traveling a certain distance and being removed, or by an undulating type belt moving the potatoes in and out of the oil; the oil flow carrying the potatoes along from one end of the fryer to the other. Some manufacturers use a double fry. That is, after the first fry, approximately 350–370°F, the potatoes fall onto another belt and enter another fryer at about the same temperature. There are several reasons for this; the principle one being that there is more even coloring because of the stirring of the potatoes as they fall from one belt to another.

92.4.10 Fat or Oil

The term "fat" refers to a product that is plastic at room temperature such as lard or the usual vegetable shortenings. Oils are liquid at ordinary temperatures. The terms are here used to mean the same thing. Any animal or vegetable fat or oil which does not impart an unpleasant flavor to the French fries is suitable for the purpose. Different processors use different oils. However, peanut oil, cotton-seed oil, or mixtures of vegetable oils including some amount of soybean oil are also used. Lard, which is hog fat imparts a flavor to the French fries that is particularly desirable to some people. Soybean oil in large amounts may impart a flavor that is usually disliked. Hydrogenated lard is tasteless.

One of the biggest difficulties in proper frying is to maintain the fat or oil in good condition. Fats and oils deteriorate rapidly with the addition of water under high temperature, also when in contact with bronze or brass fittings. When the frying oil deteriorates it darkens in color, develops unpleasant odors which are imparted to the product. Dark

bits of burnt carbon maybe deposited on the French fries giving them an unpleasant appearance, Quality control people often use the amount of free fatty acid present in the oil as an indication of degree of deterioration. A range in the area of 0.4–1.0% is regarded as normal.

Potatoes lose up to 30–40% of their weight, principally water during frying. Water is removed from the oil by a partial vacuum created by the upward draft in the hood and attaching stack covering the frying vat. Condensation from the hood is carried away by troughs along the edge of the hood. The tendency to deteriorate may be checked by eliminating bronze or brass fitting, adjusting size of fryer to volume of potatoes, using oil that will stand the highest temperature in the system, and by adding new oil from time to time.

In the better processing methods the amount of oil used is very small and is usually heated by super-heated steam in a heat exchanger rather than by direct flame. This keeps the oil in all parts of the system well below the scorching point. Usually the oil is filtered continually to remove charred materials and is thus kept clean.

92.4.11 Time and Temperature

There are many variants to be considered in determining the time and temperature of the fry. Potatoes of high specific gravity require less time to lose their excess moisture than those of low specific gravity. Different varieties of potatoes and potatoes in different conditions with respect to reducing sugars may require different cooks to attain a uniform degree of color. Certain markets seem to want potatoes fried much lighter in color than do other markets. French fries packed for institutional use, where an additional fry is to be given by the users, are usually fried to a much lighter color than are retail packs where the cooking is usually completed by the oven method. These light colored fries are usually designated as "oil-blanched" or "par-fried."

Probably the most satisfactory means of arriving at the correct time and temperature for frying is to actually fry representative sample batches of each new load. If samples come out too dark either the time or the temperature, or both, of the cook may be reduced; if too light they may be increased. In most plants quality control people watch the color of the fries as they leave the fryer, both for overall color and for uniformity of color and recommend suitable adjustments of the process. These recommendations may be based on experience or on actual color plates or models which are provided as guides for the operators. The USDA color standards may be used for this purpose. Immediately after coming from the fryer heat may be applied to drive off excess surface oil. In many plants the potatoes are cooled quickly after the fry by a blast of air. This air blast maybe designed to blow off the outer oil which clings to the hot potatoes.

92.4.12 Packaging

Packaging is usually accomplished by automatic machinery which places the proper amount of the French fries into each package. The packages are usually weighed individually and adjusted for exact weight. This packaging operation may take place before freezing or, if belt freezing is used, after the potatoes emerge from the freezer. The resulting end product of the belt freezing method is easier to handle because the units separate

easily where as the plate frozen product may emerge as one solid unit. Broken units are more common when the product is belt frozen.

92.5 INSPECTION DURING PACKING OPERATIONS

The basic principles of in-plant inspection apply in general to inspection during manufacture. Processing operations as outlined above and as observed in the plant will suggest observation to be made and the best points to make them.

Good sanitation, particularly with respect to conveyors, belts, cutting machines, and machinery that comes in contact with cut potatoes is particularly important because yeasts, molds, and bacteria thrive in a potato-water medium and odors develop quickly. Also, there may be a build-up of oil or grease between fryer and packaging lines.

Samples checked for color at the discharge end of the fryers will indicate whether the potatoes are in proper condition for frying. Samples taken over the last shaker and just prior to packaging can be checked for defects (including defectives per pound). Cooking tests should be made as soon as practical after freezing in order to develop all the information necessary for the In-plant Inspection Report.

92.6 INSPECTING THE PRODUCT

92.6.1 Sample Unit Size

Any change in sample unit sizes from those specified in the standards changes the probability of the lot of passing or failing the intended grade. The size of the sample unit used is, therefore, very important. The sizes are:

- In Retail Type – 16 ounces of product selected either from a production line or from one or more market packages.
- In Institutional Type – 32 ounces of product selected either from a production line or from one market package.

CAUTION: Make every effort to obtain a representative sample. French fries, particularly strip styles, tend to stratify themselves with vibration. Therefore, try to take from the full depth on the belt or package rather than from the top. Often a "sweep" across the entire width of a belt would be better than from just one spot.

92.6.2 Initial Fry Color, Types, Styles, and Length Designations

These items provide much needed standardized language for trading since these terms – previously widely used – were subject to much individual interpretation. Accurate identification of the fry color, type, style, and length designation is very important. They should be reported on all certificates.

92.6.2.1 Fry Color Definitions. Color changes caused by frying require special consideration. Keep in mind the following definitions: (1) Fry color refers to the color change which occurs in the potato units solely because of the initial frying or oil blanch process.

(2) Fry color of the individual units is ascertained by comparing them with the USDA Color Standards for Frozen French Fried Potatoes. The range of color includes the "color space" up to but not including, the next darkest color. (3) Fry color of the sample unit is the range of colors that occur in the frozen product before any additional heating.

92.6.2.2 Fry Color Designations. Fry color designation of a sample unit is the fry color designation appropriate to the ranges specified in the Standards.

The USDA color standards referenced are a series of colors which depict changes that occur solely because of the frying process. They are numbers 0, 1, 2, 3, and 4.

These designations are further amplified as follows: USDA No. 0 in the color standards has no browning caused by frying. The background colors of all these illustrations is yellow. Background colors of potato strips are usually basically white. They may be creamy-white, yellow-white, or any other characteristic color (see Table 92.1).

92.6.2.3 Refry Color Definitions. "Refry color" means the actual color of a potato unit after heating – either deep frying or in an oven.

"Refry color of the sample unit" is the range of colors that are present after heating in preparation for grading.

"Refry color designation" is the color designation which may be given to the sample unit after heating. The appropriate criterion for this designation is given in Refry or (after heating) Color Range Guide in later discussion.

92.6.2.4 Types. Many plants pack primarily for retail and others primarily for the institutional market. Some pack an identical product for both types. For retail, however, the fry process usually has progressed to the extent that there is some color change and sufficient oil is retained that French fried potatoes of characteristic texture may be prepared by heating the product in an oven. For institutional use the units are usually processed very lightly, resulting in little color change and often not enough oil

TABLE 92.1 USDA Colors.

USDA Color	Optional Fry Color Designation	Application to a Sample Unit
No. 0	Extra light	A sample unit may be designated "extra light" if almost all of the units have no fry color at the edges as in USDA No. 0.
No. 1	Light	A sample unit may be designated "light" if most of the potato units are lighter than USDA Color No. 2.
No. 2	Medium light	A sample unit may be designated "medium light" if most of the potato units are lighter than USDA Color No. 3 but may include Color No. 1.
No. 3	Medium	A sample unit may be designated "medium" if most of the potato units are darker than USDA Color No. 2 and may further range in color as dark as Color No. 4.
No. 4	Dark	A sample unit may be designated "dark" if most of the potato units are darker than USDA Color No. 3. This designation may contain units similar to No. 4, and darker. Sample units designated No. 4 "dark" fry color are not allowed in Grade A.

retention for proper preparation in an oven. This is often referred to as oil blanched or par fried.

The determination of type is based on intended use. You must make this determination on the information available to you.

Guidelines for this decision are as follows:

1. Small packages (5 pounds or less) which are labeled or marked as is customary or required for retail sales, and particularly those bearing official USDA marks, are considered to be "retail type." Five-pound packages which are so marked, however, may be considered to be institutional type if declared by the applicant to be intended for such use.
2. Packages of any size which are not labeled or marked as is customary or required for retail sales and display are considered to be "institutional type" unless specifically declared to be retail by the applicant for inspection.
3. If the product is unpackaged, as on belts or in tote bins, or if the packaging does not indicate the intended use, it is considered to be "retail type" and the retail type defective allowances apply. Such a lot, however, may be considered to be "institutional type" if so requested by the applicant.

92.6.2.5 Styles

Strips. This style should be designated as either:

- Straight cut;
- Straight cut-shoestring;
- Crinkle cut.

The cross-sectional dimensions of the strips are also important to the buyer. Because of the nature of the product these are not very uniform. Designate the cross-sections, therefore, as "approximate" and to $\frac{1}{8}$ inches as approximately $\frac{5}{8} \times \frac{5}{8}$ inch, or $\frac{5}{8} \times \frac{3}{4}$ inch, and so on. The cross-sectional dimension of crinkle cut strips are normally measured from "hill" to "valley."

Slices, Dices, Rissolé, Other. See chapter on "Frozen vegetables and product description."

92.6.2.6 Length Designations. (Applies only to strips). Length in French fries is closely related to quality and value for many purposes. "Extra long", for example, is usually considered a "premium" pack for institutional use. It is seldom packed for retail since it presents difficulties in packaging in retail size containers, and often requires sizing of the uncut potatoes. "Long" is packed in both retail and institutional type and is often considered a "premium" pack for retail. "Medium" is the usual retail size.

With the exception of "short" lengths which are specifically excluded from U.S. Grade A, the length of units is not considered to be a factor of quality under the U.S. standards. "Short" lengths may, however, be designated "U.S. Grade A Short" if the strips meet the other requirements of U.S. Grade A.

The lengths designated in the standards are intended to provide workable and much needed definitions for terms which are regularly used in trading.

Determining the length. The length designation may be determined readily by isolating the strips that are 3 inches in length or longer and those that are less than 2 inches in length. The percentages "2 inches in length or longer" and "3 inches in length or longer" may be readily calculated. Chips, slivers, pieces, and strips which are less than $\frac{1}{2}$ inch in length are not considered in the total count.

See USDA File Code 130-A-75 for description and scale drawing of the Vegetable Strip Sizer, a very effective device for sizing the strips.

Minimum equipment for inspecting frozen French fried potatoes:

1. Grading scale
2. Large flat trays
3. Ruler (size and length grading plate)
4. Percentage calculator
5. Authorized visual USDA color standards for frozen French fried potatoes
6. Vegetable strip sizer
7. Oven of suitable type, or deep frying equipment.

92.6.3 Preparation of Sample

The factors of color and defects are partially evaluated before the product is heated. Often when a package is opened there is a film of frost on the units which masks the color or if storage conditions have not been good there may be a crust of ice or a heavy coating of ice crystals. If there is any appreciable condition of frost, ice crystals, or icing in the sample, thaw until the condition disappears to the extent that the color may be properly evaluated. Icing is usually not serious; however, the thawing of the sample in the oven may add enough moisture to the potatoes so that they are soggy when cooked, also cause an explosion when put into hot frying oil (see Texture).

The sample should be examined for color designation using the USDA Color Standards as a guide, as discussed under Color.

92.7 QUALITY EVALUATION

92.7.1 Grade Factors Which are not Scored

92.7.1.1 Flavor. The flavor of French fried potatoes is affected by the conditions of the potatoes with respect to sugar or sunburn, the condition of the fat or oil used, and, to a certain extent, by the variety of the potatoes, the type of soil, and climatic conditions; whether or not certain insecticides have been applied to the growing potatoes.

Good flavor is required in Grades A and A Short and at least reasonably good flavor in Grade B. Sweetness, bitterness, rancidity of oil, and pronounced scorched or caramelized flavor and odors are the usual reasons for lowering the evaluation of flavor from "good" to only "reasonably good." Any definitely objectionable flavors or odors would be cause for lowering the grade of the product to Substandard. After the product has been heated in a suitable manner, taste and smell it and classify its flavor as "good," "reasonably good," or "poor."

92.7.2 Color Designation of a Sample Unit

The exact color of good quality potatoes varies considerably because of varietal differences, physical differences, types of fat used, areas of production, and other causes. It also varies because of the amount of color change induced by the frying process. These values are important to buyers because certain markets and certain important customers have strong preferences as to the lightness or darkness of the brown coloring.

Two separate and distinct color determinations are required:

1. Classifying the "fry color of the sample unit" as to its value (that is, its lightness or darkness) in order to establish the proper fry color designations; and
2. Evaluation and assigning the score points for color in compliance with the standards, giving consideration to color changes in the refried product.

Grade A, Good Color (27–30 points). This color is bright and typical of the product and meets the uniformity of fry color given for:

- No. 0 – extra light
- No. 1 – light
- No. 2 – medium light
- No. 3 – medium

and meets the uniformity of refry color given in the Refry Color Range Guide.

Grade B Reasonably Good Color (24–26 points) (Limiting Rule). This color must be characteristic of French fried potatoes – not dull or off color. It may exceed the fry color variation given for any of the USDA colors – including No. 4 – dark. After heating, the variation in the refry color may exceed those indicated in the guide but may not seriously detract from the appearance of the product.

Substandard (0–23 points) (Limiting Rule). Lots that darken quickly – before the interiors are cooked, or very irregular would fall into this classification.

92.7.3 Uniformity of Size and Symmetry

Uniformity of length of normal shaped strips is not considered under this factor. Consideration is given to the effect of any "chips" as defined on the appearance of the product and the percent by count of "small pieces," "slivers," and/or "irregular pieces." In assigning score points be guided by the following:

Grade A

20 points – almost no chips and/or:

Strips – no more than 5% of small pieces, slivers, and/or irregular pieces.

Other styles – almost perfect uniformity in size and shape of the units.

18 points – chips present but not to materially detract from appearance, and/or;

Strips – more than 5–15% of small pieces, slivers, and/or irregular pieces.

Other styles – high degree of uniformity in the size and shape of the units.

19 points – by interpolation.

Grade B

17 points – chips present materially detract and/or:

Strips – more than 15–20% small pieces, slivers, and/or irregular pieces.

Other styles – reasonably uniform in size and shape.

16 points – chips present that approach serious appearance, and/or:

Strips – more than 20–30% small pieces, slivers, and/or irregular pieces.

Other styles – variation in the size and shape of the units detracts noticeably from the appearance of the product.

92.7.4 Defects

Defects are carefully defined in the standards as: Insignificant imperfections, minor defects, and major defects.

Defectives are potato units affected with defects – as defined in the standards as "minor defective" or "major defective." It is defectives rather than defects which are scored against.

92.7.4.1 Considerations. For each grade three separate types of deficiencies are considered under this factor. While the principal consideration is major and minor defectives, all three must be considered in assigning the scores for the sample units. They are:

1. The total effect of all faults which might be present whether specifically mentioned. This is the "overall clause." Among such are extraneous materials, insignificant imperfections, and carbon specks or defects (as defined), and obnoxious blemishes which are much worse in appearance than usual major defect;
2. The effect of any carbon specks on the appearance of the product; and
3. The allowances for minor and major defectives as specified in Tables 92.2 and 92.3 of the standards.

92.7.4.2 Defect Tables in the Standards. Defectives allowed in these tables are not averages. Sample units that fail the applicable requirement are allowable in the sample only as regular deviants.

92.7.5 Assigning the Score for Defects

92.7.5.1 Procedure

1. Segregate the minor and major defectives in the sample unit and record them on the score sheet as (1) total (major and minor) and (2) major.
2. Assign a tentative score for defects as indicated by the following guide.

TABLE 92.2 Standards – All Styles Except Shoestrings and Dices.

Grade	Point	Defective	Possible Combinations of Defectives			
Retail type						
A	20	Total	0–3			
		Major	0			
	19	Total	4–5	1–3		
		Major	0	1		
	18	Total	4–5			
		Major	1			
B	17	Total	6–9	6–9	2–5	
		Major	0	1	2	
	16	Total	6–9			
		Major	2			
Institutional type						
A	20	Total	0–6	1–4		
		Major	0	1		
	19	Total	7–18	5–18	2–12	3–8
		Major	0	1	2	3
	18	Total	13–18	9–18	4–18	
		Major	2	3	4	
B	17	Total	19–28	5–23	6–18	
		Major	0–4	5	6	
	16	Total	24–28	19–28	7–28	
		Major	5	6	7–8	

TABLE 92.3 Standards – Shoestring, Strips, and Dices.

Grade	Point	Defective	Possible Combinations of Defectives			
Retail type						
A	20	Total	0–5	1–2		
		Major	0	1		
	19	Total	6–9	3–5	2–5	
		Major	0	1	2	
	18	Total	6–9	6–9		
		Major	2	1		
B	17	Total	10–18	3–15	4–8	
		Major	0–2	3	4	
	16	Total	16–18	9–18	5–18	
		Major	3	4	5	
Institutional type						
A	20	Total	0–10	1–8		
		Major	0	1–2		
	19	Total	11–28	9–28	3–21	5–18
		Major	0	1–2	3–4	5–6
	18	Total	22–28	19–28	7–28	
		Major	3–4	5–6	7–8	
B	17	Total	29–36	9–30		
		Major	0–8	9–10		
	16	Total	31–36	11–36		
		Major	9–12	11–12		

Groups are inclusive i.e., $\frac{3-5}{1}$ *means* $\frac{3}{1}\ \frac{4}{1}$ *or* $\frac{5}{1}\ \frac{Total}{Major}$.

3. Adjust the score point if appropriate by giving consideration to the "overall clause" and the effect of any carbon specks present. This becomes the defect score for the sample unit.

Guide for assigning tentative score for defects – subject to adjustment for "overall clause" and for carbon specks.

92.7.5.2 Texture. Texture is evaluated within three minutes after heating the product as specified, and while it is well above room temperature.

Heating the Product

OVEN METHOD. The method of reheating specified in the standards is similar to that employed by the housewife. The crumpled foil is placed in the bottom of the pan in order to prevent excessive burning of the potatoes where they touch the metal pan. Fifteen minutes at 400° is a minimum for most potatoes. The time depends on the size of the units, the sugar content, and the type of oven – whether gas or electric – the number of samples in the oven, and how well it is ventilated. Trial runs are usually necessary to determine the proper time to cook any lot of potatoes in the available equipment. Potatoes are properly cooked when the interior of the largest units has lost the raw potato taste. This method should be used when it is obvious that the product is intended for home use and cooking directions call for the oven method. Exceptions may be made when test runs have shown that the "deep fat" method gives results comparable to the oven method on the particular potatoes.

DEEP FAT METHOD. Frozen French fried potatoes prepared for institutional use usually have a lighter fry color than those prepared for the retail trade. This is because the institutions using these potatoes will give them a short fry in oil. This additional fry can be adjusted in time and temperature so that the finished French fries will have the desired color. This desired color may be light or fairly dark depending upon the preference of the cooks. Also the directions on some retail packages provide for an additional cook in hot oil rather than an oven cook. For this reason provision is made in the U.S. standards for heating the product by any other method which will give comparable results.

Deep fat frying is probably preferred for inspection use because of the speed with which the samples can be run. It should always be used where the product is light in color and/or obviously intended for institutional use. Where large numbers of samples are to be inspected a deep fat fryer of the type marketed for household use and provided with an automatic heat control is very useful. If only an occasional sample is to be inspected equally good results maybe obtained by using a small stew pan with a wire dipper. With this equipment it is necessary to have an emersion thermometer capable of registering up to 600°F. Also, new automatic frying pans can be obtained with heat control units.

Heat at least 100 units to determine the score for character. The temperature of the oil is very important. The temperature must be high during the entire refry time or the results will be in error. One-hundred units in a very large tank such as may be available in-plant inspection would not lower the temperature significantly. With a quart or pint of oil only a few units can be fried at a time without lowering the oil temperature. Good texture varies somewhat with the varieties used and the area of production. It

may vary from a somewhat cheese-like, very fine grained texture to a coarse-grained almost powdery texture.

Usual Variations from Acceptable Texture

SOGGINESS. As the name implies this refers to a wet pasty or mushy condition either loaded with water or oil. It may be a basic characteristic of the potatoes or it may be induced by frying at too low a temperature. Often only a portion of the potato becomes soggy. Both the amount of the unit affected and the degree of sogginess must be considered in estimating the effect on texture. Score the unit only if 50% of its length (or less if very objectionable) is so affected.

HARDNESS. Interior portions that are very firm, sometimes oily to touch and raw in taste even if well cooked. Often – as with sogginess – only a portion of a strip or slice is hard. Score such units only if 50% (or less if very objectionable) of its length is so affected.

PULL AWAY. Interior portion of a strip that has withdrawn from the outer shell voiding $\frac{1}{3}$ of the cross sectional area of a regular strip or $\frac{2}{3}$ of the cross section of a shoestring.

CRISP OUTER SURFACE. Really crisp outer surfaces is a texture fault in any grade. A slight crispness is expected in Grade A and the surfaces may be slightly hard or slightly tough in Grade B. Keep in mind that excessive cooking will increase the crispness of the outer surfaces.

SUGARY ENDS. A unit that has a dark and often soft rubbery end, caused by excess sugar.

EXCESSIVE OILINESS. For reasons which are not always explainable an unusual amount of oil is sometimes retained by the fries. It is very objectionable to buyers as it affects the texture adversely. Excessive oiliness can often be detected by the feel of the units prior to the heating. If excessive oiliness does not disappear with normal preparation, lower the texture score to reflect this condition.

Score Points. The exact score points to assign requires careful preparation of the sample. Consider all the factors affecting texture and assign scores as indicated in the following guide: Scoring Procedure: Heat 100 strips to determine the Texture Score. The number of points deducted from a possible 30 points will depend on the overall excellence of the sample. Consideration must also be given for those units in a sample that have a soggy or hard texture, or show pull away, or have excessively oily outer surfaces. Sugary ends not serious enough to be considered defects would fall into this category. The sample shall be practically free of such units to score in the Grade A range. Percentages ranging from 0–10% by count depending on the seriousness of the defective units, are acceptable in this grade.

Prepared French fried potatoes that are scored 24–26 points for texture must be reasonably free from soggy or hard texture, pull away, or sugary ends, or those which do not have a crisp outer surface.

Score 26 points if there are 11–15% by count of these scorable units, or if the units with slightly soggy or hard interior portions, or soft or slightly hard exterior surfaces materially affect the overall appearance or eating quality of the product.

Score 25 points if there are 16–20% by count of the scoreable units and 24 points if there are 21–25% by count.

93

Paprika Production: Current Processing Techniques and Emerging Technologies

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93.1 Introduction 1031
93.1.1 Description of Carotenoids as Components Responsible for Fruit Color 1032
93.2 Industrial Processing of Red Pepper for Production of Paprika 1034
93.2.1 Current Techniques Applied for Dehydration of Red Pepper Fruits 1035
93.2.2 Emerging Technologies and Processing Strategies Applied for Dehydration of Red Pepper Fruits 1036
93.2.3 Milling 1036
93.3 Effect of Processing and Storage on the Carotenoid Content 1037
93.3.1 Processing 1037
93.3.2 Storage 1039
93.4 Determination of Quality of Paprika 1040
References 1041

93.1 INTRODUCTION

Since the discovery of the New World, red pepper fruits spread widely to the rest of the world. Today, this fruit is grown for commercial purposes in the United States, Brazil, India, Taiwan, South Africa, Zimbabwe, and through all Europe, with Hungary and Spain as the most representative producers of paprika. Pepper plant (*Capsicum* genus)

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belongs to the *Solanaceae* family as well as potato, tomato, tobacco, and petunia, and even coffee. This genus includes 22 wild species and five domesticated species: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*, with *C. annuum* and *C. frutescens* being the most economically important. Fruits of these plants are used in the manufacture of selected commercial products known for their color and pungency respectively, mainly paprika and paprika oleoresins. *Capsicum frutescens* L. plant is cultivated in the tropics and warmer regions of the United States, and its fruits are used to provide pungency in tabasco sauce. *Capsicum annuum* L. plant is extensively cultivated, producing fruits that vary in length, color, and pungency depending upon the cultivar. The nonpungent cultivars are used for direct consumption, either fresh or dried, whole or ground. They are also widely used as color additives to improve or provide that organoleptic attribute in other foodstuffs.

Fruits of these plants are berries, with different shapes (rounded or elongated) and size (few grams up to 250 g) depending on the variety. Seeds are straw-colored and placed on the placenta. During ripening, color of fruits changes from green to red due to a massive de novo biosynthesis of carotenoid pigments, which is accompanied by a catabolism of the chlorophylls originally present in the unripe fruits.

Coloring power is the main characteristic in determining commercial value for paprika and derived products, and this property is directly correlated with the carotenoid content. Selection and breeding of varieties have been performed in order to increase carotenoid content of fruits (Hornero-Méndez and others 2000, 2002). Other selection criteria are fruitful ability of plants, resistance to rot, and finally, grouped ripeness and plant morphology to facilitate mechanical harvest. As mentioned before, selection of fruits and economic evaluation of their processed products will be made in terms of coloring capacity, and these attributes are measured with the appropriate methodology. The compounds responsible for color of these fruits are carotenoids as discussed in the next section.

93.1.1 Description of Carotenoids as Components Responsible for Fruit Color

The fruits of *C. annuum* owe their intense red color to carotenoid pigments that are synthesized massively during fruit ripening. Carotenoids are isoprenoid compounds with an extensive system of conjugated double bonds (polyene chain) which is responsible for their physical and chemical properties. This characteristic structure is the primary cause for their lipophilic behavior, as well as for their distinctive light-absorbing properties and therefore of their coloring capacity. In the case of red pepper fruits, the main carotenoid pigments responsible for the final red color of the fruits are capsanthin, capsorubin, and capsanthin-5,6-epoxide, which are almost exclusive to the genus *Capsicum* (Davies and others 1970; Mínguez-Mosquera and others 1984; Mínguez-Mosquera and Hornero-Méndez 1994a).

Carotenoids are essentially C40 terpenoid compounds formed by the condensation of eight isoprene units. Their biosynthesis is an important branch of the complex isoprenoid pathway. The basic carotene structure (i.e., lycopene) undergoes several structural modifications namely cyclization, hydroxylation, epoxidation and rearrangement, giving way to the great variety of carotenoids in Nature (more than 650) (Britton 1998). During ripening of the pepper, there is a spectacular *de novo* biosynthesis of carotenoid pigments.

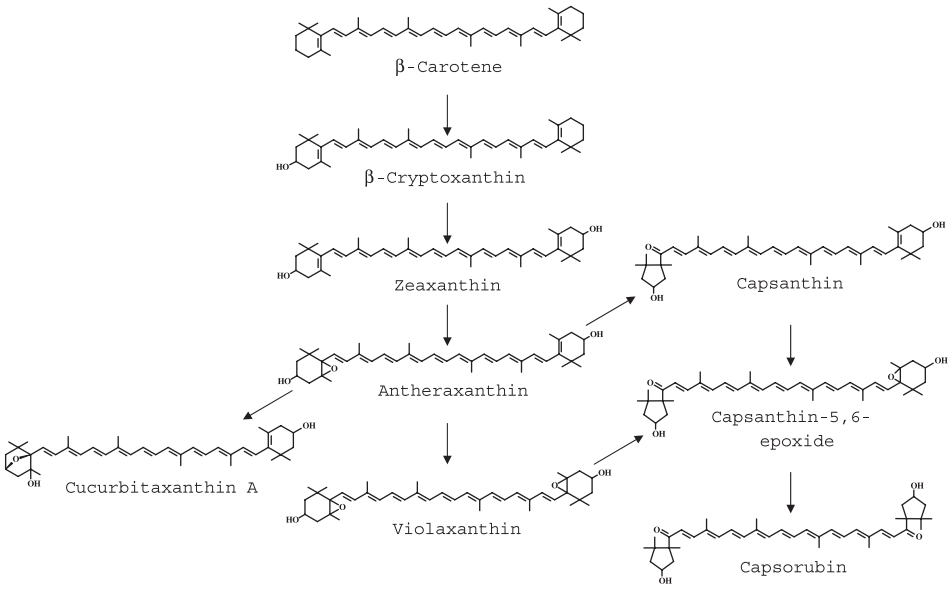

Figure 93.1 *Scheme of the basic biosynthetic pathway of carotenoids in red pepper fruits (Capsicum annuum L.). Red fraction comprises capsanthin, capsanthin-5,6-epoxide and capsorubin. Yellow fraction includes rest of carotenoid pigments.*

Figure 93.1 shows the basic route for the biosynthesis of carotenoids found in red pepper fruits. All the carotenoid pigments present in the pepper contain nine conjugated double bonds in the central polyene chain. The presence of different end groups (β, ε, κ, 3-hydroxy-5,6-epoxide), changes the chromophore properties of each pigment, allowing them to be classified in two isochromic families: red (R) and yellow (Y). The red fraction contains capsanthin, capsanthin-5,6-epoxide, and capsorubin, while the yellow fraction comprises the rest of the pigments (zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene, and cucurbitaxanthin A), which act as precursors of the former.

An important characteristic of the group of xanthophylls is that they are esterified with fatty acids, a fact that takes place during ripening of pepper (Camara and Monéger 1978). Several functions or benefits result from the esterification of xanthophylls, such as the increase of liposolubility, enhancing the stability of these pigments towards enzymatic and nonenzymatic oxidative reactions, and that they may be used as indicators of ripeness index (Hornero-Méndez and Mínguez-Mosquera 2000). Yellow xanthophylls are mainly esterified with unsaturated fatty acids like oleic and linoleic while saturated fatty acids esterify the red xanthophylls (Biacs and others 1989; Mínguez-Mosquera and Hornero-Méndez 1994b). Besides the coloring capacity, two properties of carotenoids must be remarked. First, β-carotene and its isomeric forms, and β-cryptoxanthin show provitamin A activity. Second, all carotenoids perform antioxidant capacity, independently of the provitamin A activity. The antioxidant capacity of carotenoids could be displayed in the food where they are added or in human body, once these pigments are ingested. The antioxidant properties displayed by the carotenoids are also indicative that these pigments will be easily degraded under oxidative conditions, such as increased light and temperature,

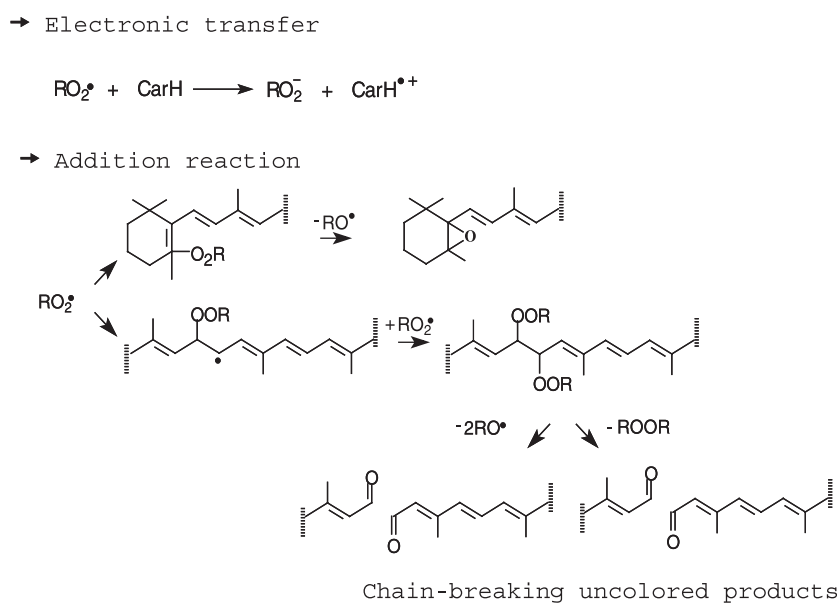

Figure 93.2 *Radical reaction pathways affecting the polyene chain of carotenoids that modify and disrupt the coloring properties.*

presence of free radicals, and reactive oxygen, through which carotenoids have been demonstrated to be effective quenchers (Foote and Denny 1968). Frequently, these conditions are promoted during fruit processing. The interaction between carotenoids and prooxidants takes place mainly through the polyene chain (Fig. 93.2) and any reaction or excess of energy of the environment affecting this chain will modify coloring properties of the pigment. Consequently, coloring capacity is diminished through oxidative processes and thus economic value of the product will decrease. This correlation is considered during processing of red pepper fruits for paprika, as the unit operations applied may considerably increase the progress of oxidative reactions affecting carotenoid concentration of fruits and their later stability. Hence, the suitability of the type of processing and conditions applied will be assessed in terms of minimal degradation of the carotenoid content and its maximal stability during the storage of the final product.

93.2 INDUSTRIAL PROCESSING OF RED PEPPER FOR PRODUCTION OF PAPRIKA

Paprika is a fine, brilliant, highly colored powder resulting from the application of two unit operations to mature red pepper fruits, dehydration and milling. Dehydration of fruits reduces the water content from 70–85% to 10%. This operation increases the shelf-life of fruits from days to months and allows application of the second unit operation: milling. This size reduction is necessary to homogenize the color of the dry fruits batches, and facilitate its use as colorant.

Dehydration could be considered as the main processing step in paprika manufacturing because conditions (temperature and time regime) may produce profound changes in the

carotenoid content as a consequence of the degradative reactions that either directly or indirectly degrade pigment profile of fruits. And these changes may be denoted immediately after processing or triggered during storage of the product. Therefore, in this processing step is where more technological innovations have been implemented, nowadays even more, with the trend to reduce impact of thermal processing on food constituents. Current techniques and emerging technologies of dehydration are described in the next sections.

93.2.1 Current Techniques Applied for Dehydration of Red Pepper Fruits

Traditionally, two conventional dehydration techniques have been used, via direct exposure of fruits to sunlight in open air, a characteristic process of Murcia (Spain) or in drying chambers where the heat source is the burning of oak logs, the traditional process carried out in La Vera (Spain). The first form of traditional drying has been employed in Spain, Turkey, and Tunisia. But the rain damage, dust and insect contamination, and quality of dry peppers, affected by oxidative degradation promoted by sunlight (Mínguez-Mosquera and others 1996), are serious drawbacks that have made this easy technique to be disregarded. However, economically and environmentally, this dehydration method is very attractive and some efforts are now conducted to improve the technique. The main innovation is to perform the dehydration under shelter, in greenhouses and some trials have been made to obtain mathematical models for moisture evolution of fruits. Even the possibility of introducing forced convection in the greenhouses has been explored. These studies are still performed only from the physical point of view, so they should be complemented with the analysis of the quality of the product (Passamia and Saravia 1997; Ratti and Mujumdar 1997; Condori and Saravia 1998).

Industrial processes, with alternative heat sources have replaced the solar dehydration technique. Traditionally, the combustion of oak logs has been the heating source to increase temperature of air going into drying chambers. Heat is generated in the first floor of the cottages and the fruits are piled on the second one, the surface of which is a wooden lattice that allows hot air and smoke to circulate through the mass of fruits. This method of dehydration is characterized by continuous rises and falls in temperature associated with the combustion of wood, a manual process without technical equipments. Thus, the temperature–time profile is very irregular, but with a cyclic period of rise and fall of temperature coinciding with the fire up and fire down during the 7–10 days of dehydration (Mínguez-Mosquera and others 1996; Pérez-Gálvez and others 2001). The final product, dry husks, presents flavor features highly prized and appreciated by both the consumer and food producers, as the flavor will provide unique organoleptic characteristics to a meal or the product where this kind of paprika is included as well as the coloring properties.

However, this low temperature–time regime has two special considerations: variability, and a lack of modern technology to control this traditional process. As a result, conventional industrial dryers become more suitable for the dehydration step. The temperature–time regime is more uniform, the residence time of fruits is reduced from days to 4–6 h, and moisture content of the dry product is even lower (4–6%). Moreover, the process is frequently made in a continuous flow fashion, using tunnel dryers (Chung and others 1992; Levy and others 1995). The hot air (60–80°C) circulates through the tunnel while fresh fruits, deposited on trays, move intermittently through it (McGaw and others 2001). One improvement is the use of vacuum dryers that would considerably

reduce the thermal stress, and the temperature–time regime could be reduced, decreasing pressure, using vacuum roller dryer (Zhang and others 2003).

93.2.2 Emerging Technologies and Processing Strategies Applied for Dehydration of Red Pepper Fruits

Although preprocessing techniques of raw material before dehydration are not generally used, some studies show that application of pretreatments to fresh fruits reduces temperature–time regime by increasing the rate of water transfer (Lazarides and others 1999). Combination of techniques such as washing, chemical peeling, cutting, and steam blanching considerably decreases processing time and consequently the degree of thermal stress (Doymaz and Pala 2002).

Undoubtedly, osmotic dehydration has become one of the reference techniques to be applied for reducing the negative impact of conventional convective dryers, thus improving the organoleptic properties of the fruits, mainly the color, but also original composition in other components, such as vitamin C, so that nutritional value observed in fresh fruits could be preserved. This technique consists on placing the fresh fruits into highly concentrated salt or sugar solutions. Control variables are composition of the dehydration solution, immersion time and temperature of the pretreatment. The concentration gradient makes water from fruits to diffuse into the solution. The dehydration level of the fruit material is later reduced to the desired level by application of a conventional dehydration technique. This preprocessing step decreases the energy requirements of the dehydration operation.

Osmotic dehydration may also be combined with high intensity electric field pulses that disrupt the cell material and produce pore formation that modifies skin permeability properties, or with application of high hydrostatic pressure that affects cell wall structures. Application of these preprocessing techniques considerably reduces temperature–time regime in convective dryers to reach the dehydration level of fruits (Ade-Omowaye and others 2002).

Novel thermal processes like infrared, microwave, ohmic, and inductive radio frequency and high heat infusion techniques might also be considered as alternatives to conventional dehydration. In general, these drying technologies enhance the diffusion of heat and mass. Microwave and radio frequency dehydration methods start to dry the product internally and the heat diffuses to the surface, enabling increased drying rates and improved product quality.

One strategy that could be implemented is to perform dehydration of fruits in an inert atmosphere or processing under vacuum that will diminish the oxidative effects over pigments, vitamins, and aroma, and therefore maintaining product quality features (Ramesh and others 1999).

93.2.3 Milling

The size reduction of dry fruits is achieved by milling. This process has the following profiles:

- Homogenizes the coloring capacity of different batches,
- Facilitates transport and storage, dosage of product in formulation of foodstuffs, and application of further processing for obtaining paprika oleoresins.

The dry husk is milled with the seeds of the fruit that enhances color surface and brightness. Seeds percentage in paprika is 30–35% and they act as a dilution factor because they do not contribute to any carotenoid pigmentation (Mínguez-Mosquera and others 1993; Pérez-Gálvez and others 1999a). A common particle size after milling is 40 μm.

Two types of mills are usually utilized: ball and hammer mills. Ball mills consist of a slowly rotating, horizontal steel cylinder, half filled with steel balls (2.5–15 cm in diameter). The final particle size depends on the speed of rotation and on the size of the balls. Hammer mills consist of a horizontal or vertical cylindrical chamber lined with a steel breaker plate and contains a high-speed rotor fitted with hammers along its length. The material is broken apart by impact forces as the hammers drive it against the breaker plate.

93.3 EFFECT OF PROCESSING AND STORAGE ON THE CAROTENOID CONTENT

93.3.1 Processing

The quality of processed fruits and vegetables is governed by a major factor. The original organoleptic and nutritional properties of the raw material should remain after processing. For red peppers, this means that the carotenoid content, that gives the nutritional value in paprika, and its main organoleptic property, the color, should be retained as close as possible to the initial levels. In addition, carotenoids provide this fruit with significant health attributes besides the color, for example, provitamin A level and antioxidant properties. Preserving the color during processing means that those nutritional characteristics are also preserved, a correlation schemed in Figure 93.3. Like in other fruits, vitamin C retention in red peppers after processing could be used as indicative of processing quality (Tijskens and others 1979) although carotenoids are the main markers (Mínguez-Mosquera and others 1992).

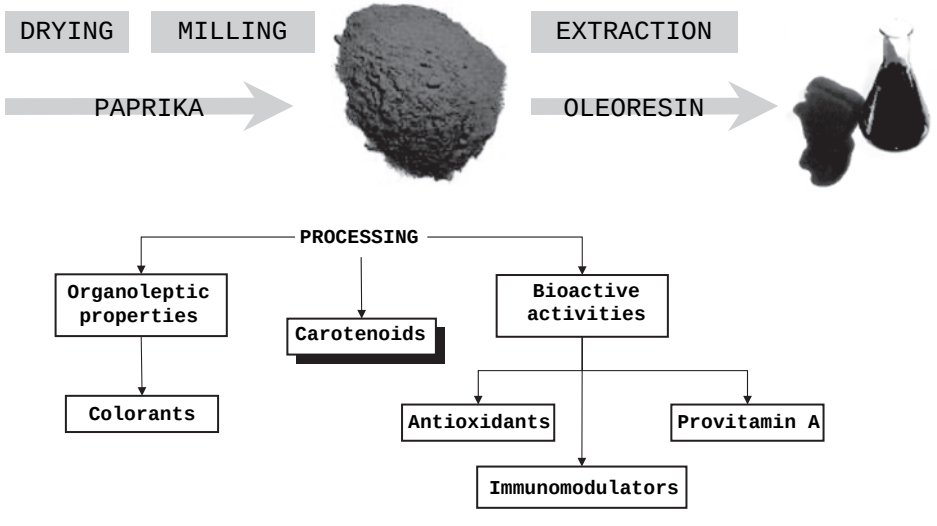

Figure 93.3 *Unit operations applied for manufacturing of paprika and paprika oleoresins and consideration of the effect of those processing steps on properties of carotenoids.*

Dehydration parameters, time, temperature, and moisture of fruits will define the outcome of carotenoid content. These pigments are sensitive to those factors that promote oxidative reactions and the thermal stress to which the fruits are subjected to during dehydration. They will determine the extent of changes in the carotenoid composition of the dry product (Mínguez-Mosquera and others 2000).

Current technologies applied for dehydration of processing have resolved the equation "temperature and time residence" in different means. While conventional industrial processes diminish the residence time by increasing the temperature, the traditional process extends the residence time and the processing temperature is considerably reduced. These different approaches will have different consequences in the color quality of the dry product obtained.

Dehydration conditions in convective dryers, (high temperature–time regime), facilitate nonenzymatic browning reactions and carotenoid loss (Lee and Kim 1989). Percentage of loss will depend on processing conditions. A report of Mínguez-Mosquera and others (1994a) shows a 25% of loss on the total carotenoid content when fresh fruits were oven-dried. A subsequent work (Mínguez-Mosquera and Hornero-Méndez 1994c) shows a 16% of loss after dehydration at 60°C during 8 h.

The traditional drying process carried out in the La Vera region (Spain) is milder, with a mean temperature of 40°C during the 7–10 days of processing. Several reports on this way of drying confirm that color quality remains unaltered in most cases. Of course, thermal stress and oxidative reactions take place. However, mild temperature conditions and low drying rates allow fruits to keep high moisture levels during the first phase of dehydration. This increases the over ripening of the fruits, activating the anabolic pathway for carotenoids (Mínguez-Mosquera and others 1994b, 2000; Pérez-Gálvez and others 2004). The biosynthesis of carotenoids compensates loss of pigments due to degradative reactions yielding a dry product with a total carotenoid content very similar to that of the fresh product. There is a correspondence between temperature profile, catabolism and anabolism of carotenoids, and moisture content (Pérez-Gálvez and others 2004, 2005). These results help us to establish, during the dehydration process, the operation conditions that will modify the carotenoid content towards anabolic and catabolic reactions.

Biosynthetic reactions are, therefore, the metabolic reply to the thermoxidative stress. This anabolic capacity makes possible a balance between anabolism and catabolism, hence keeping the characteristics of fruits. Moreover, it has been observed that the anabolic capacity is increased when fruits are harvested before total ripeness because they keep metabolically active and have more capacity to modulate the stress. The concept of cyclic time–temperature varying profile of this traditional processing is reproduced during dehydration of some food products to enhance quality and reduce drying time. The need for a lower drying time by using temperature–time varying profiles has been mathematically demonstrated by Devahastin and Mujumdar (1999). Chua and others (2000a) have used time-varying air temperature profiles to reduce the change in color parameters. The application of temperature fluctuations during dehydration also reduces the loss on other valuable components, such as vitamin C (Chua and others 2000b). Effect on quality during drying and degradation profile during storage after application of this dehydration strategy should be extensively studied.

Effect of novel technologies and pretreatments on the carotenoid concentration of red pepper fruits is a subject of research in recent years. The effort made to establish processing conditions for the application of osmotic predehydration, high intensity electric pulses and high hydrostatic pressure, and the analysis performed of physical variables

must be complemented with the effect of such techniques on chemical parameters of composition of valuable components, mainly carotenoids. The physical changes produced in cellular structure of fruits during osmotic dehydration facilitates the process, but still a variable percentage of loss is observed. The work of Ade-Omowaye and others (2002) reports a variable loss, ranging from 80% to 55% after the osmotic dehydration at different temperatures. It was observed that due to the permeability, a carotenoid fraction is lost by diffusing to the osmotic solution. Combination of osmotic dehydration with high intensity electric pulses diminished the losses. Composition of osmotic solution has been reported to have some effect on color characteristics of osmosed peppers (Torreggiani and others 1995).

High temperatures used in the steam blanching produce losses of carotenoids when this pretreatment is combined with the subsequent conventional hot air dehydration. Alternative use of inert gas atmosphere has shown to reduce carotenoid losses when blanched red peppers were dried with hot air. In any case, losses are lower in comparison with those achieved with hot air dehydration without any pretreatment or modification of the atmosphere (Ramesh and others 1999).

Few reports discuss over the effect of milling on the carotenoid content of dry fruits. Degradation arises from temperature increase due to friction and bruising from the mill hammers or balls. In the region of Murcia (Spain), production of paprika under PDO (Protected Designation of Origin) regulation specifies that temperature milling must not increase above 45°C. Residence time in mills takes no more than 3 h but losses of 10–15% have been reported (Mínguez-Mosquera and others 2000).

93.3.2 Storage

Stability of food components during storage is not only a direct consequence of storage conditions but also the result of content in compounds that may act as antioxidants and the presence of promoters of degradative reactions, and finally derived from processing parameters applied for manufacturing the final product. Degradation of the carotenoid profile during processing could be used as a marker of stability of the product at the store, as well as oxidation of the fatty acid profile, which would give the paprika undesirable flavor that would making the product inappropriate for trade and consumption (Biacs and others 1992). Additionally, it must be pointed out that although degradative reactions may initiate during processing, their consequences would not be noticeable immediately, but later during storage (Malchev and others 1982).

To avoid degradation during storage, different common strategies should be established. Vacuum packaging of paprika in red plastic bags avoids direct contact to air and light. Storage at low temperatures and appropriate water activities is also advisable as stated by Lee and others (1992). Like in other foodstuffs, progress of autoxidative reactions, which are responsible for the degradation of carotenoid pigments, could be delayed in paprika by addition of antioxidants (ascorbic acid, tocopherols, herbs extracts). These methods avoid exogenously the oxidation potential of the sample, which is mainly based on the polyunsaturated fatty acid profile of the paprika (Pérez-Gálvez and others 1999a,b) and promoted by excessive thermal stress during paprika processing (Malchev and others 1982, 1989), inappropriate storage conditions or a combination of both issues.

Addition of antioxidants is a common practice to increase stability of foodstuffs. In the case of paprika, the ascorbic acid content in the raw material (the red pepper fruits) could

be considered high (3–5 mg/g dry fruit), but during processing the fruit losses the main part of it (Daood and others 1996; Pérez-Gálvez and others 2004). Thus, addition of ascorbic acid to improve antioxidant capability of dry material could be considered as a reconstitution technique. This is useful in the sight of results described in some studies (Biacs and others 1992; Carvajal and others 1997). Other antioxidants used to improve coloring stability of paprika have been tocopherols, and rosemary extract, which showed different coloring protection properties depending on storage conditions. At temperatures higher than 25°C, the tocopherols display a marked protective effect, while at 5°C the rosemary extract has the strongest antioxidant action.

The protective effect of added antioxidants seems to depend on conditions used to store paprika like relative humidity and temperature. High humidity levels provide the proper environment where hydrophilic antioxidants (like ascorbic acid) could perform their antioxidant activity (Kanner and others 1978). When temperature increases, then the activity of the lipophilic antioxidants becomes the key as autoxidative reactions take place at higher rates. To achieve a wide range of antioxidant activity in different storage conditions, more than one antioxidant should be added (Ladrón-de-Guevara and others 2002).

In any case, addition of antioxidants is avoided due to labeling restrictions, mainly when the paprika is commercialized under PDO regulation. Moreover, use of other strategies to improve color stability should be more appropriate instead of reconstitution or adding antioxidants, in order to maintain authenticity of the product. One of these strategies could be the endogenous modification of the oxidation potential. If the lipophilic matrix of the food is prone to oxidation (high content of polyunsaturated fatty acids such as in paprika), changing that composition by a monounsaturated profile could minimize oxidative susceptibility (Pérez-Gálvez and others 1999b, 2000). This could be achieved by application of a breeding program where cultivars with a less polyunsaturated fatty acid profile should be selected.

The technological innovation in drying techniques and the study of the effect of dehydration on paprika compounds should not stop at the characterization of product immediately after processing, but also analyses during storage should be performed. Application of different techniques could change the behavior of stability as this highly depends on temperature–time regimes of dehydration (Ramesh and others 2001; Ade-Omowaye and others 2003). Therefore, quality of the product, in terms of carotenoid retention, should not only be based on coloring capacity but also on the stability of that quality attribute during storage. This point will be discussed below.

93.4 DETERMINATION OF QUALITY OF PAPRIKA

Measurement of color is the reference for determining economical value of paprika. The higher coloring capacity the higher price. Several straightforward standard procedures for determination of coloring capacity have been developed to obtain values of that capacity that allows ranking of different batches of paprika. Hence, "Standard" method determines the color value by measuring the absorbance of a 1/10,000 solution of a known weight of paprika in acetone at 462 nm, multiplying the result by 66,000 and divided by sample weight to obtain a standard color value (Guenther 1948). ASTA method consists of a color extraction of a weighed paprika sample with 100 mL acetone during 24 h. An aliquot of that solution (10 mL) is diluted again to 100 mL, and a portion of the diluted solution is used for the spectrophotometric measurement at 460 nm. ASTA units are

calculated by multiplying the absorbance by 164 and divided by sample weight (ASTA 1986).

Other spectrophotometric approaches have been achieved to obtain a more approximated carotenoid composition of paprika, as the abovementioned methodologies only give a global value of color provided by all carotenoids of the sample. The method developed by Hornero-Méndez and Mínguez-Mosquera (2001) allows a simultaneous quantification of the red and yellow fractions with low error in the determination and lower time analysis in comparison with that of chromatographic methods. But quality determination of a sample based on a spectrophotometric measurement gives only an isolated static idea of it without possibilities of prediction of its evolution with time. Measurement of promoter components of degradative reactions such as peroxides could help to infer the potential stability of paprika.

Hornero-Méndez and others (2001) have developed a simple spectrophotometric method for determination of peroxide value in paprika samples that may help to that purpose. Individual determination of carotenoid composition of sample by using chromatographic methods provides a more detailed information about the carotenoid profile, the provitamin A activity, and the extent of degradative reactions affecting yellow and red isochromic fractions. Ratio between the two fractions should remain invariable if the processing does not affect the two fractions, or to the same extent if it affects initial ratio value. This ratio has been proposed as a useful quality index either for the fruits or their processed products (Mínguez-Mosquera and Fernández-Díez 1981; Mínguez-Mosquera and others 1984, 1992). It is known that yellow fraction is more prone to oxidation than the red one, so a high value of the ratio should indicate that degradative reactions have taken place. Other predictive tools for assessing stability of carotenoids during storage have also been established (Jarén-Galán and others 1999). On the other hand, the red to yellow ratio may vary if commercial pigments, like canthaxanthin and β-apo-8′-carotenal, or other carotenoid rich sources like tomato, are added to enhance the red hue. In this case the application of HPLC methodologies has been demonstrated to succeed on this fraud detection (Mínguez-Mosquera and others 1995).

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94

Pickles Manufacturing in the United States: Quality Assurance and Establishment Inspection

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94.1 Quality Assurance 1046
94.1.1 Introduction 1046
94.1.2 Product Description 1046
94.1.3 Styles of Pickles 1046
94.1.4 Types of Pack 1047
94.1.4.1 Cured Type 1047
94.1.4.2 Fresh-Pack Type 1048
94.1.4.3 Refrigerated Type 1049
94.1.5 Sizes of Whole Pickles 1049
94.1.6 Definitions of Terms 1050
94.1.6.1 Analytical Definitions 1050
94.1.6.2 Blemished 1051
94.1.6.3 Color 1051
94.1.6.4 Shape 1051
94.1.6.5 Flavor and Odor 1052

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Handbook of Food Products Manufacturing. Edited by Y. H. Hui

94.1.7 Recommended Fill of Container 1053
94.1.8 Quantity of Pickle Ingredient 1053
94.1.8.1 Drained Weight/Volume 1054
94.1.9 Sample Unit Size 1054
94.1.10 Grades 1054
94.1.11 Factors of Quality 1055
94.1.12 Requirements for Grades 1055
94.2 Establishment Inspection 1056
94.2.1 Pickle Products 1057
94.2.1.1 Examination of Raw Materials Used in Relish 1057
94.2.1.2 Class 1058
94.2.2 Peppers 1059
94.2.3 When Insects are Found in a Sweetening Tank 1061
94.2.4 Other Points of Interest 1061
94.2.5 Sample Collection 1061
94.2.5.1 Bulk Salt Stock for Filth 1061
94.2.5.2 Finished Pickle Product – All Types 1062

94.1 QUALITY ASSURANCE

94.1.1 Introduction

This section is not designed to explain how pickles are manufactured in the United States. Rather, it is designed to show you the critical factors you should look for in assuring the quality of your pickles. The information has been modified from a document issued by the United States Department of Agriculture: *United States Standards for Grades of Pickles*. Consult the original document for complete details.

This section contains 11 tables of reference data, numbering 94.1 to 94.11 and they do not appear in the same order as the text.

94.1.2 Product Description

Pickles means the product prepared entirely or predominantly from cucumbers (*Cucumis sativus* L). Clean, sound ingredients are used that may or may not have been previously subjected to fermentation and curing in a salt brine. The product is prepared and preserved through natural or controlled fermentation or by direct addition of vinegar to an equilibrated pH of 4.6 or below. The equilibrated pH value must be maintained for the storage life of the product. The product may be further preserved by pasteurization with heat, or refrigeration and may contain other vegetables, nutritive sweeteners, seasonings, flavorings, spices, and other permissible ingredients defined by the U.S. Food and Drug Administration (FDA). The product is packed in commercially suitable containers to assure preservation.

94.1.3 Styles of Pickles

1. Whole style means the pickles are whole and are relatively uniform in diameter as indicated in a latter discussion (Table 94.2).
2. Whole, mixed sizes style means the pickles are whole pickles of mixed sizes.

3. Sliced lengthwise style means the pickles are cut longitudinally into halves, quarters, or other triangular shapes (spears, strips, or fingers), or otherwise into units with parallel surfaces with or without ends removed.
4. Sliced crosswise, crosscut, or waffle cut style means the pickles are cut into slices transversely to the longitudinal axis. The cut surfaces may have flat-parallel or corrugated-parallel surfaces.
5. Cut style means the pickles are cut into chunks or pieces that are of various sizes and shapes.
6. Relish style means finely cut or finely chopped pickles containing no less than 60% of cucumber ingredient and may contain other vegetable ingredients (cauliflower, onions, pepper, tomatoes, cabbage, olives, mustard, or any other suitable vegetable).

94.1.4 Types of Pack

94.1.4.1 Cured Type. The pickles are cured by natural or controlled fermentation ina salt brine solution and may contain the dill herb or extracts thereof. The pickle ingredient may be partially desalted. The pickles may be further processed or preserved by the addition of vinegar and may contain other ingredients (spices, flavorings, firming and preserving agents) that constitute the characteristics of the particular type of pickle. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. The characteristics of the various types of cured pickles are as follows:

1. Dill pickles (natural or genuine) are cucumbers that are cured in a brine solution with dill herb and other flavoring agents.
2. Dill pickles (processed) are brine-cured pickles that have undergone a freshening process and are packed in a vinegar solution with dill flavoring and other flavoring agents.
3. Sour pickles are cured pickles that are packed in a vinegar solution with or without spices.
4. Sweet pickles and mild sweet pickles are cured pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s).
5. Sour mixed pickles are cured pickles that are packed in a vinegar solution. The pickles may be of any style or combination of styles other than relish and may contain other vegetable ingredients as outlined in Table 94.1 or any other suitable vegetable.
6. Sweet mixed pickles and mild sweet mixed pickles are cured pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s). The pickles may be of any style or combination of styles other than relish and may contain other vegetable ingredients as outlined in Table 94.1 or any other suitable vegetable.
7. Sour mustard pickles or sour chow chow pickles are cured pickles of the same styles and ingredients as sour mixed pickles except the pickles are packed in a prepared mustard sauce of proper consistency with or without spices and flavorings.
8. Sweet mustard pickles or sweet chow chow pickles are cured pickles of the same styles and ingredients as sweet mixed pickles except the pickles are packed in a sweetened, prepared mustard sauce of proper consistency with or without spices and flavorings.

TABLE 94.1 Proportions of Pickle Ingredients in Certain Types and Styles.

	Cured; Fresh-pack; and Refrigerated Types	
	Sour Mixed; Sweet Mixed; and Mild Sweet Mixed; Sour Mustard or Sour Chow Chow; Sweet Mustard or Sweet Chow Chow	Sour Pickle Relish; Sweet Pickle Relish; Dill Relish; Hamburger Relish; Mustard Relish
Pickle Ingredients and Styles	Percent by Weight of Drained Weight of Product	
Cucumbers, any style other than relish	60–80%	–
Cucumbers, chopped or finely cut	–	60–100%
Cauliflower pieces	10–30%	–
Cauliflower, chopped of finely cut	–	30% maximum (optional)
Onions, whole (maximum diameter of $1-\frac{1}{4}$ inches), sliced or cut	5–12%	–
Onions chopped or finely cut	–	12% maximum (optional)
Green tomatoes, whole or pieces	10% maximum (optional)	–
Green tomatoes, chopped or finely cut	–	10% maximum (optional)
Red, green, or yellow peppers. or pimientos, cut, finely cut or pieces	Optional	Optional
Celery	Optional	Optional
Cabbage	Optional	Optional
Olives	Optional	Optional
Tomato paste	Optional	Required in hamburger relish
Mustard or prepared mustard	Required in chow chow and mustard pickles	Required in mustard relish, optional in hamburger relish

9. Sour pickle relish consists of finely cut or chopped cured pickles that are packed in a vinegar solution. Sour pickle relish may contain other chopped or finely cut vegetable ingredients as listed in Table 94.1, and may contain a stabilizer such as a starch or gum.
10. Sweet pickle relish and mild sweet pickle relish are finely cut or chopped cured pickles that are packed in a vinegar solution with a suitable nutritive sweetening ingredient(s). Sweet pickle relish and mild sweet pickle relish may contain other chopped or finely cut vegetable ingredients as listed in Table 94.1 and may contain a stabilizer such as a starch or gum.
11. Hamburger relish consists of relish style pickles and other chopped or finely cut vegetable ingredients as listed in Table 94.1 with tomato product added.
12. Mustard relish consists of sweet pickle relish with mustard and other chopped or finely cut vegetable ingredients as listed in Table 94.1.
13. Dill relish consists of relish style pickles containing dill flavoring and other chopped or finely cut vegetable ingredients as listed in Table 94.1.

94.1.4.2 Fresh-Pack Type. The pickles are prepared from uncured, unfermented cucumbers and are packed in a vinegar solution with other ingredients to produce the characteristics of the particular type of pack. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. In addition, the pickles are sufficiently

processed by heat to assure preservation of the product in hermetically sealed containers. The distinguishing characteristics of the various types of fresh-pack pickles areas follows:

1. Fresh-pack dill pickles are pickles that are packed in a vinegar solution with dill flavoring.
2. Fresh-pack sweetened dill pickles are pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s) and dill flavoring.
3. Fresh-pack sweetened dill relish consists of finely cut or chopped pickles packed in a vinegar solution with suitable nutritive sweetening ingredient(s) and dill flavoring. The relish may contain other finely cut or chopped vegetable ingredients as listed in Table 94.1.
4. Fresh-pack sweet pickles and fresh-pack mild sweet pickles are pickles that are packed in a vinegar solution with nutritive sweetening ingredient(s).
5. Fresh-pack sweet pickle relish and fresh-pack mild sweet pickle relish consists of finely cut or chopped pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s). The relish may contain other finely cut or chopped vegetable ingredients as listed in Table 94.1.
6. Fresh-pack hamburger relish consists of relish style pickles and other chopped or finely cut vegetable ingredients as listed in Table 94.1 with tomato product added.
7. Fresh-pack mustard relish consists of sweet pickle relish with mustard and other chopped or finely cut vegetable ingredients as listed in Table 94.1.
8. Fresh-pack dill relish consists of relish style pickles containing dill flavoring and other chopped or finely cut vegetable ingredients as listed in Table 94.1.
9. Fresh-pack dietetic pickles are pickles that are packed with or without the addition of sweetening ingredient(s), salt (NaCl), or other suitable ingredient(s) as declared and permitted under FDA regulations.

94.1.4.3 Refrigerated Type. The pickles are prepared from fresh cucumbers and are packed in a vinegar solution with other ingredients to produce the fresh crisp characteristic of refrigerated type. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. They are stored, distributed, and displayed under refrigeration and may or may not contain one or more chemical preservatives. The various types of refrigerated pickles are the same as the types listed for fresh-pack type in earlier discussion with respect to ingredients except that they conform to the requirements for refrigerated type.

94.1.5 Sizes of Whole Pickles

Sizes of whole pickles are based on the diameter and the relationship of diameter to the count per gallon. Size designations, applicable counts, and diameters are outlined in Table 94.2 of this subpart. The diameter of a whole cucumber is the shortest diameter at the greatest circumference measured at right angles to the longitudinal axis of the cucumber.

94.1.6 Definitions of Terms

For an interpretation of this standard, some definitions of terms are:

TABLE 94.2 Sizes of Processed Whole Pickles.

		Glass			Metal	
Word Designation	Diameter	1 qt.	$\frac{1}{2}$ gal	1 gal	No. 10	No. 12 (1 gal)
Midget	19 mm (0.75 in) or less	67 or more	135 or more	270 or more	202 or more	270 or more
Small gherkin	Up to 2.4 cm (0.94 in)	33–66	67–134	135–269	101–201	135–269
Large gherkin	Up to 2.7 cm (1.06 in)	16–32	32–66	65–134	48–100	65–134
Small	Over 2.7 cm (1.06 in) but not over 3.5 cm (1.38 in)	10–15	20–31	40–64	30–47	40–64
Medium	Over 3.5 cm (1.38 in) but not over 3.8 cm (1.50 in)	6–9	13–19	26–39	19–29	26–39
Large	Over 3.8 cm (1.50 in) but not over 4.4 cm (1.73 in)	4–5	9–13	18–25	13–18	18–25
Extra large	Over 4.4 cm (1.73 in)	2–3	6–8	12–17	9–12	12–17

94.1.6.1 Analytical Definitions. Analytical definitions refer to analytical laboratory requirements.

1. Acid means total acidity calculated as acetic acid in accordance with the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC).
2. Brix value (Brix) means the percent sugar, by weight, corrected to 20°C (68°F), as determined with a sugar scale Brix hydrometer or other instrument that gives equivalent results.
3. Degrees Baumé means the density of the packing medium determined with a Baume' hydrometer (modulus 145) corrected to 20°C (68°F).
4. Equalization means the natural (osmotic) or simulated blending between the soluble solids of the pickle ingredient and the packing medium.

Natural equalization means equalization brought about after a period of time has elapsed after processing as follows. Sweetened pickles are considered to be equalized 15 days or more after processing. If the pickles have been sweetened in a tank prior to packing, the pickles will be considered equalized 15 days after the sweetening process began. Sour and dill pickles are considered to be equalized 10 days or more after processing.

Simulated equalization means a method of simulating equalization by comminuting the finished product in a mechanical blender, filtering the suspended material from the comminuted mixture and making the required tests on the filtrate.

5. Total chlorides or salt means the salt content expressed as grams NaCl (sodium chloride) per 100 mL packing medium; except that total chlorides in mustard pickles and chow is determined and expressed in grams NaCl per 100 g of product.

94.1.6.2 Blemished. Blemished means any unit that is affected by discoloration, pathological injury, insect injury, or similar causes to the extent that the appearance or edibility of the product is adversely affected:

1. Slightly – those blemishes which detract only slightly from the appearance of the unit;
2. Seriously – those blemishes which strongly detract from the appearance or edibility of the unit.

94.1.6.3 Color.

1. Good color in cured type means the typical skin color of the pickles ranges from a translucent light green to dark green and is practically free from bleached areas. Not more than 10%, by weight, of the pickles may vary markedly from such typical color. In mixed pickles, chow chow pickles, and pickle relish, all of the ingredients possess apractically uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
2. Good color in fresh-pack and refrigerated types means the typical skin color of the pickles ranges from an opaque yellow-green to green. Not more than 15%, by weight, of the pickles may vary markedly from such typical color. In pickle relish, all of the ingredients possess a good uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
3. Reasonably good color in cured type means the typical skin color of the pickles ranges from light green to dark green and is reasonably free from bleached areas. Not more than 25%, by weight, of the pickles may vary markedly from such typical color. In mixed pickles, chow chow pickles, and pickle relish, all of the ingredients possess a reasonably uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
4. Reasonably good color in fresh-pack and refrigerated types means the typical skin color of the pickles ranges from light yellow-green to green. Not more than 30%, by weight, of the pickles may vary markedly from such typical color. In pickle relish, all of the ingredients possess a good, fairly uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
5. Poor color in all types of pickles means the pickles fail to meet the requirements for good or reasonably good color for the respective type.

94.1.6.4 Shape. (Also see the definition of misshapen.) Crooked pickles mean whole pickles that are curved at an angle greater than 60° as illustrated in Figure 94.1.

Curved pickles mean whole pickles that are curved at an angle of 35–60° when measured as illustrated by Figure 94.2.

Diameter in whole style means the shortest diameter measured transversely to the longitudinal axis at the greatest circumference of the pickle. Diameter in cross-cut style is the shortest diameter of the largest cut surface.

Defect means an imperfection such as curved, misshapen, mechanically damaged, discolored, and other imperfection that affects the appearance or edibility of the product.

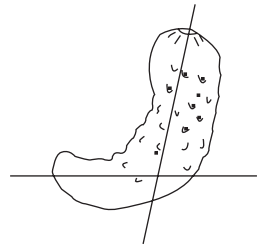

Figure 94.1 *Crooked pickles (also see the definition of misshapen).*

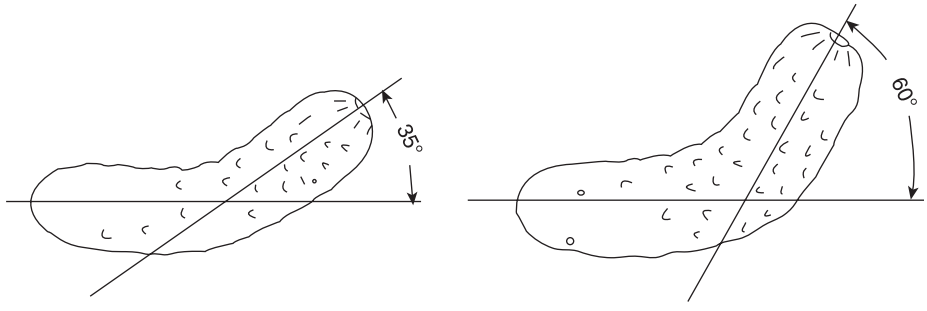

Figure 94.2 *Curved pickles.*

End cut means a pickle unit intended for crosscut (sliced crosswise) style that has only one cut surface.

Extraneous vegetable material (EVM) means any harmless vegetable material, other than stems, that is not normally part of the pickle ingredient. EVM such as leaves or other vegetable material not associated with proper pickle preparation or packaging is considered a defect if it affects the appearance or edibility of the product either:

Slightly – Practically free of EVM and does not more than slightly affect the appearance or edibility; or

Materially – Reasonably free of EVM and does not more than materially affect the appearance or edibility.

94.1.6.5 Flavor and Odor

1. Good flavor and odor means characteristic flavor and odor (e.g., characteristic dill flavor or the like) typical of properly processed pickles, for the type, that is free from objectionable flavor and odor of any kind.
2. Reasonably good flavor and odor means flavor and odor that may be lacking in characteristic flavor for the type but is free from objectionable flavor and odor.
3. Poor flavor and odor means flavor and odor that fails to meet the requirements for good or reasonably good flavor and odor.

Length in sliced lengthwise style means the longest straight measurement at the approximate longitudinal axis.

Mechanical damage refers to crushed or broken units that affect the appearance of the units. In relish, mechanical damage refers to units which are poorly cut and have a ragged or torn appearance.

Misshapen pickles mean whole pickles that are crooked or otherwise deformed (such as nubbins). Also see the definition for crooked pickles.

Nubbin is a misshapen pickle that is not cylindrical in form, is short and stubby, or is not well developed.

Texture means the firmness, crispness, and condition of the pickles and any other vegetable ingredient(s) and freedom from large seeds, detached seeds, and tough skins that may be present. The following terms also relate to texture:

1. Hollow centers in whole style, means the pickles, when cut transversely to the longitudinal axis, are missing $\frac{1}{3}$ or more of the seed cavity.
2. Soft, shriveled, and slippery units refers to pickles that are wrinkled, not crisp, slick, flabby, or lack firmness.
3. Good texture means the pickle units have been properly processed and possess a texture that is firm and crisp.
4. Reasonably good texture means the pickle units have been properly processed but lack some of the firmness and crispness that is characteristic for the style and type of pack.
5. Poor texture means the pickle units do not meet the requirements for good or reasonably good texture.

Uniformity of size (relish style only).

1. Practically uniform in size means the size of the units may vary moderately in size but not to the extent that the appearance or the eating quality is seriously affected.
2. Poor uniformity of size means the units fail the requirements for practically uniform.

Unit means one whole, half, slice, or piece of pickle as applicable for the style.

Units missing $\frac{1}{3}$ or more of the seed cavity in crosscut style means pickles that have lost a substantial portion of the seed cavity such as a crosscut unit missing $\frac{1}{3}$ or more of the seed cavity portion.

94.1.7 Recommended Fill of Container

The recommended fill of container is not a factor of quality for the purposes of these grades. Each container of pickles should be filled with pickle ingredient, as full as practicable, without impairment of quality. The product and packing medium should occupy not less than 90% of the total capacity of the container.

94.1.8 Quantity of Pickle Ingredient

The recommended minimum quantity of pickle ingredient is designated as the percentage of the declared volume of product in the container for all items except pickle relish. Minimum quantity of pickle relish is designated as a relationship of the drained weight of the pickle ingredient to the declared volume of the container. The minimum quantities recommended in Tables 94.3 and 94.4 are not factors of quality for the purposes of these grades.

TABLE 94.3 Recommended Pickle Ingredients: All Styles Except Relish.

Type of Pack	Minimum Fill (Volume)
Cured	55%
Fresh-pack	57%
Refrigerated	57%

TABLE 94.4 Recommended Drained Weight to Container Volume, Relish.

Type of Pack	Minimum Fill (Weight/Volume)
Cured	
Sweet	92%
Other than sweet	88%
Fresh-pack	
Sweet	85%
Other than sweet	80%

The percent volume of pickle ingredient is determined for all styles, except relish, by one of the following methods in accordance with the procedures prescribed by the USDA:

1. Direct displacement (overflow-can method);
2. Displacement in a graduated cylinder;
3. Measurement of pickle liquid;
4. Any other method that gives equivalent results and is approved by the USDA.

94.1.8.1 Drained Weight/Volume. The percent weight/volume (w/v) of relish shown in Table 94.4, is determined as follows: The drained weight of pickle relish of all types is determined by emptying the contents of the container upon a U.S. Standard No. 8 circular sieve of proper diameter containing eight meshes to the inch (0.0937 inch $\pm$ 3%, square openings) so as to distribute the product evenly, inclining the sieve slightly to facilitate drainage, and allowing to drain for 2 min. The drained weight is the weight of the sieve and the pickles less the weight of the dry sieve. A sieve 8 inches in diameter is used for 1 quart and smaller size containers and a sieve 12 inches in diameter is used for containers larger than 1 quart in size.

94.1.9 Sample Unit Size

For all styles of pickles and types of pack, the sample unit used in analyzing the quality factors is the entire contents of the container unless otherwise specified in CFR Part 7.

94.1.10 Grades

1. U.S. Grade A is the quality of pickles that meets the applicable requirements of Tables 94.5–94.11 and scores not less than 90 points.

TABLE 94.5 Analytical Requirements: [a]Cured Type Pickles, all Styles.

	Maximum		Minimum	
	Total Acidity Expressed as Acetic Acid g/100 mL, Unless Otherwise Indicated	Total Chlorides Expressed as NaCl g/100 mL, Unless Otherwise Indicated	Degrees Brix	Degrees Baumé
Cured type all styles		–	–	–
Dills (natural, genuine or processed)	1.1	5.0	–	–
Sour, sour mixed, dill pickle relish, sour relish	2.7	5.0		–
Sweet whole, sweet mixed, and sweet relish	2.7	3.0	27.0	15.0
Mild sweet, mild sweet mixed, mild sweet relish	–	–	20.0	12.0
Sour mustard or sour chow chow	2.7[b]	3.0[b]	–	–
Sweet mustard or sweet chow chow	2.7[b]	3.0[b]	28.0	15.5
Fresh-pack and refrigerated types, all styles	–	–	–	–
Dills and sweetened dills	1.1	4.25	–	–
Sweetened dill relish	1.1	4.25	–	–
Sweet and mild sweet relish	1.65	2.75	–	–
Sweet and mild sweet pickles	1.65	2.75	–	–
Dietetic	–	–	–	–

[a]All pickle products must have an equilibrated pH of 4.6 or below.
[b]Expressed as g/100 g.

2. U.S. Grade B is the quality of pickles that meets the applicable requirements of Tables 94.6–94.11 and scores not less than 80 points.
3. Substandard is the quality of pickles that fails the requirements of U.S. Grade B.

94.1.11 Factors of Quality

The grade of pickles is based on the following quality factors:

1. Analytical requirements in Table 94.5
2. Flavor and odor
3. Color
4. Uniformity of size
5. Defects
6. Texture

94.1.12 Requirements for Grades

See Tables 94.5–94.11.

TABLE 94.6 Quality Requirements: Whole Style Pickles.

	Grade A		Grade B	
	Maximum (By Count)	Score	Maximum (By Count)	Score
Flavor and odor	Good		Reasonably good[a]	
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size[b]		18–20		16–17
Diameter variation				
Midget and Gherkin [over 8 mm (0.31 in)]	10%		20%	
Small and Medium [over 10 mm (0.39 in)]	10%		20%	
Large and extra large [over 12 mm (0.47 in)]	10%		20%	
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Curved pickles	10%		20%	
Misshapen	5%		15%	
Mechanical damage	10%		15%	
Attached stems [over 2.5 cm (0.98 in)]	10%		20%	
Extraneous vegetable material (EVM)	Practically free		Reasonably free[a]	
Texture	Good	27–30	Reasonably good[a]	24–26
Large seeds, detached seeds, tough skins	Practically free			
Soft, shriveled, and slippery units	5%		10%	
Hollow centers	15%		25%	
Total score (minimum)	90 points		80 points	

[a]Cannot be graded above U.S. Grade B, regardless of the total score.
[b]Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

94.2 ESTABLISHMENT INSPECTION

The United States Food and Drug Administration has issued guidelines for the inspection of a pickles processing plant. Some of the information is provided in this chapter. The quality control officer in such a plant should use the information to implement its in-plant inspection procedure.

The information is presented in the teacher/student format for ease of reference.

1. Direct special attention to the following areas when inspecting these types of food establishments. If the establishment is producing acidified Fresh Pack Pickles, determine if the establishment is complying with the requirements of 21 CFR 114, Acidified Foods.
2. Salt Stations and Salt Stock Tanks.
3. Insects which breed in decomposed pickles or other decaying organic matter such as the lesser or little house fly, the latrine fly, the house fly, the rat-tailed maggot, and

TABLE 94.7 Quality Requirements: Whole Style Pickles, Mixed Sizes.

	Grade A		Grade B	
	Maximum (By Count)	Score	Maximum (By Count)	Score
Flavor and Odor	Good		Reasonably good[a]	
Color	Good	18–20	Reasonably good[a]	16–17
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Curved pickles	10%		20%	
Misshapen	5%		15%	
Mechanical damage	10%		15%	
Attached stems [over 2.5 cm (0.98 in)]	10%		20%	
Extraneous vegetable material (EVM)	Practically free		Reasonably free[a]	
Texture	Good	27–30	Reasonably good[a]	24–26
Large seeds, detached seeds, tough skins	Practically free		Reasonably free	
Soft, shriveled, and slippery units	5%		10%	
Hollow centers	15%		25%	
Total score (minimum)[b]	90 points		80 points	

[a]Cannot be graded above U.S. Grade B, regardless of the total score.
[b]Total score is adjusted by dividing the total score by 0.80 to allow for the absence of the quality factor of uniformity of size in whole mixed sizes style.

drosophila are of major sanitary significance. Examine 25% of the tanks for insect filth.

4. "Mill run" salt may be used but workers should not walk in the salt.
5. Tanks should be skimmed daily for debris and insects and the skimmings should be properly disposed of.
6. Newly salted stock ferments – scum growth should be removed regularly and disposed of so that insects are not attracted.

94.2.1 Pickle Products

94.2.1.1 Examination of Raw Materials Used in Relish

1. Obtain the usual composition of relish in percent by weight of cucumbers as well as other ingredients to help appraise the filth load found in the sample.
2. Salt stock used for relish may consist of poor quality pickles, that is, deformed, bloated, or blemished. However, in the absence of filth, grit, or partly/wholly rotted pickles, there is no objection to their use. Mushy pickles are caused by certain pectin splitting enzymes during fermentation. Soft pickles may be invaded by bacteria and fungi, but it is frequently difficult to determine if any mold or bacteria are present by field examination.

TABLE 94.8 Quality Requirements: Sliced Lengthwise Style Pickles.

	Grade A		Grade B	
	Maximum (By Count)	Score	Maximum (By Count)	Score
Flavor and Odor	Good		Reasonably good[a]	
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size[b]		18–20		16–17
Length variation [over 2.6 cm (1.02 in)]	10%		20%	
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Mechanical damage	10%		15%	
Attached stems [over 2.5 cm (0.98 in)]	10%		20%	
Extraneous vegetable material (EVM)	Practically free		Reasonably free[a]	
Texture	Good	27–30	Reasonably good[a]	24–26
Large seeds, detached seeds, tough skins	Practically free		Reasonably free	
Soft, shriveled, and slippery units	5%		10%	
Total score (minimum)	90 points		80 points	

[a]Cannot be graded above U.S. Grade B, regardless of the total score.
[b]Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

3. Examination of cucumber salt stock for relish – when whole pickles or large pieces are used, examine a representative sample of 100 units going to chopper.
4. Segregate and list objectionable pickles as follows:

94.2.1.2 Class

	Number	*Percent*
1. With rot spots over 1/2 in.	_____	_____
2. Insect infested or damaged	_____	_____
3. Mushy or very soft	_____	_____

4. For class 1 pickles, make a further determination of the surface area of the rot spots by size; up to 1 inch; from 1 inch to half of the pickle; and over half of the pickle. Take close-up color photographs of objectionable pickles. Collect exhibits of pickles showing typical rot and insect damage.
5. Laboratory examination of mushy pickles for mold is necessary to establish if they are objectionable. If over 5% of the units are mushy, cut a thin cross-section from each pickle. Place the slices in a quart jar with water and add 20 cc formaldehyde for later examination.

TABLE 94.9 Quality Requirements: Sliced Crosswise or Crosscut Style Pickles.

	Grade A		Grade B	
	Maximum (By Count)	Score	Maximum (By Count)	Score
Flavor and Odor	Good		Reasonably good[a]	
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size[b]		18–20		16–17
Diameter [over 5.4 cm (2.13 in)]	10%		20%	
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Mechanical damage	15%		25%	
Broken pieces and end cuts	10%		15%	
Thickness over 10 mm (0.38 in)	10%		15%	
Attached Stems [over 2.5 cm (0.98 in)]	10%		15%	
Units missing 1/3 seed cavity	10%		15%	
Extraneous vegetable material (EVM)	Practically free		Reasonably free[a]	
Texture	Good	27–30	Reasonably good[a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically free		Reasonably free[a]	
Soft, shriveled, and slippery units	5%		10%	
Total score (minimum)	90 points		80 points	

[a]Cannot be graded above U.S. Grade B, regardless of the total score.
[b]Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

6. When small pieces of salt stock cucumbers, cauliflower, and peppers are used, rot determination by count is impractical. If rotten pieces are observed, collect a separate quart of each pickled vegetable. Preserve the samples with 20 cc formaldehyde. At the same time collect a sample totaling half a gallon of finished relish.

94.2.2 Peppers

1. Check for insect larvae (maggots or larvae of pepper weevil) in fresh and salt stock peppers and figure percent of infestation on a representative sample. Examine any fresh pack peppers in which infested stock was used.
2. If peppers with rot are found, evaluate in the same fashion as for cucumbers.
3. Examine vinegar storage tanks for drosophila infestation and for vinegar eels.
4. Insect filth in sweet stock pickles – insects, particularly drosophila, are attracted to the sweetening tanks, and may be found in the finished sweet pickle products.
5. Sweet brine is frequently circulated within a tank and from one tank to another dispersing insects in the circulating brine. It is sometimes difficult to estimate the

TABLE 94.10 Quality Requirements: Cut Style Pickles.

	Grade A		Grade B	
	Maximum (By Count)	Score	Maximum (By Count)	Score
Flavor and Odor	Good		Reasonably good[a]	
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size[b]		18–20		16–17
Small pieces 5 g or less	5%		10%	
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Mechanical damage	10%		15%	
Attached stems over 2.5 cm (0.98 in)	10%		15%	
Extraneous vegetable material (EVM)	Practically free		Reasonably free[a]	
Texture	Good	27–30	Reasonably good[a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically free		Reasonably free[a]	
Soft, shriveled, and slippery units	5%		10%	
Total score (minimum)	90 points		80 points	

[a]Cannot be graded above U.S. Grade B, regardless of the total score.
[b]Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B regardless of the total score.

TABLE 94.11 Quality Requirements: Relish.

	Grade A		Grade B	
	Maximum (By Weight)	Score	Maximum (By Weight)	Score
Flavor and Odor	Good		Reasonably good[a]	
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size		18–20		16–17
Overall appearance	Good		Reasonably good[a]	
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Poorly cut	10%		15%	
Loose stems over 3.0 mm (0.12 in)	10%		15%	
Extraneous vegetable material (EVM)	Practically free		Reasonably free[a]	
Texture	Good	27–30	Reasonably good[a]	24–26
Large objectionable seeds, detached seeds, & tough skins	Practically free		Reasonably free[a]	
Soft, shriveled, and slippery units	5%		10%	
Total score (minimum)	90 points		80 points	

[a]Cannot be graded above U.S. Grade B, regardless of the total score.

number of insects and parts in such circulating brine. Close examination of the inside tank walls may reveal drosophila above the brine level. These are the best indices of infestation in a tank.

94.2.3 When Insects are Found in a Sweetening Tank

1. Determine whether sweet brine in the tank is an intermediate or finishing brine and if it is circulated within the tank or between sweetening tanks.
2. If the finishing brine is used as a packing medium, determine whether it is filtered prior to use and evaluate the filtration step.
3. If sweet stock is held in infested tanks, determine anticipated date of packing.
4. Evaluate tank covers used.
5. List quantitatively, the extent of insect infestation by the collection of representative samples of filth from a definite area, for example, square feet of the walls of the tank on the sweet stock and in a specified amount of brine from different areas of the tank if the infestation is widespread. If infestation seems to be isolated, collect specimens showing the types of insects.

94.2.4 Other Points of Interest

1. Grit in pickles – excessive grit is frequently found in fresh pack pickles and in midget sweet pickles. Salt stock may occasionally contain excessive grit. If dirty cucumbers are packed, collect in-line and finished product samples.
2. Use of color and preservatives – green artificial color is sometimes used in relish without label declaration. Ascertain if the color is permitted for use and declared on the label.
3. Sorbic acid may be used in salt stock, to prevent yeast growth, and in finished pickle products, as a preservative. Where sorbic acid is present in the finished product, determine if it is declared on the label.
4. Examination of warehouse stocks – examine for evidence of spoilage, particularly in fresh pack pickles which may have been inadequately pasteurized.
5. If heavy insect infestation is found, examine 24 jars of the pickle product (other than relish) most likely to contain insects by inverting jars under strong light. Collect jars containing insects as a factory sample.

94.2.5 Sample Collection

94.2.5.1 Bulk Salt Stock for Filth. If in barrels, collect a minimum of 12 half-gallon jars of salt with their brine; two from each of six previously unopened barrels to make six duplicate subs. Collect one sub from the top and the other sub from the bottom, if possible. If in tank cars, collect a minimum of $12\frac{1}{2}$ gallon jars of salt stock and brine. If live flies are observed inside tank during sampling, note and estimate their number.

94.2.5.2 Finished Pickle Product — All Types.

Filth and Grit

Quarts and smaller jars	Minimum to collect
Up to100 cases in lot	24 jars
More than 100 cases	48 jars

Gall on jars	Minimum to collect
Up to 100 cases	12 jars
More than 100 cases	24 jars

Undeclared Color and Chemical Additives. Collect 6 quarts or 12 pints for examination.

95

Soymilk and Tofu Manufacturing

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95.1 Introduction 1064
95.2 The manufacture of Soymilk and Tofu 1065
95.2.1 Preparation of Soymilk 1065
95.2.1.1 Cleaning and Soaking 1065
95.2.1.2 Grinding 1067
95.2.1.3 Grinding Temperature 1068
95.2.1.4 Water Added During Grinding 1068
95.2.1.5 Heating and Separation 1068
95.2.2 Coagulation of Soymilk 1070
95.2.2.1 Solids Content and pH of Soymilk 1070
95.2.2.2 Types of Coagulants Available 1071
95.2.2.3 Amount of Coagulant Used 1072
95.2.2.4 Coagulation Temperature 1072
95.2.2.5 Coagulation Method 1073
95.2.2.6 Cutting of Curd 1073
95.2.3 Pressing 1074
95.3 Effect of Soybeans and Component on Soymilk and Tofu Manufacturing and Quality 1074
95.3.1 Soybean Raw Materials 1074
95.3.2 Storage Conditions 1075
95.3.3 Proteins 1076
95.3.4 Lipids and Phospholipids 1077
95.3.5 Phytic Acid 1078

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

95.3.6 Isoflavones and Saponins 1079
95.3.7 Lipoxygenases 1080
95.4 Mechanism of Tofu Curd Formation 1080
95.5 Food Quality Evaluation of Soybeans, Soymilk and Tofu: Trading and Industry Perspective 1081
95.5.1 Soybean Quality 1081
95.5.1.1 Size and Hilum Color of Soybeans 1081
95.5.1.2 Chemical Composition as Related to Food Quality 1081
95.5.1.3 Storage Changes 1082
95.5.2 Soymilk Quality 1082
95.5.3 Tofu Quality 1082
References 1083

95.1 INTRODUCTION

Availability of soymilk and tofu has a long history, since the Han Dynasty in China about 2000 years ago. It has been an integral part of the Chinese food culture, and has also spread to Korea, Japan, and several Eastern Asian countries. It is generally believed that the absence of prolonged malnutrition in the China may be due to the regular consumption of soymilk, tofu, and other soyfoods in their diet. The consumption of tofu has gradually gained acceptance in the Western culture as some of its nutritional and health benefits are now known. It is also popular among vegetarian consumers.

The main components in soymilk and tofu are water, protein, fat, and minerals. Its protein profile is not as good as milk and egg proteins but is probably the best among foods of plant origin. Soymilk is often referred to as "poor man's milk." Dieticians also consider tofu made with calcium salts as a good source of calcium. The U.S. Food and Drug Administration approved a health claim for processed foods containing soy proteins that states "consumption of 25 g soy proteins per day in conjunction with a low cholesterol diet would reduce the risk of heart disease."

The availability of tofu in various parts of the world is credited to the migration of East Asian peoples to the different countries, and also to the development of semi- to fully-automatic machines to make tofu by the Japanese. Many people can claim they can make tofu. However, making tofu with consistent quality is not that easy, even though tofu making consists of only two basic steps: preparation of soymilk and coagulation of soymilk to form the curd which is then made into various types of tofu. Many processing factors are involved in the preparation of tofu and the raw bean components are also determinant factors on the quality of tofu.

Three books contain information on soymilk and tofu making, including the The Science of Tofu (Watanabe 1997), the Tofu & Soymilk Production, the Book of Tofu, Volume 2 (Shurtleff and Aoyagi 1990), and the 'Soybeans: Chemistry, Technology and Utilization,' (Liu 1997). Two recent reviews on the science and technology of tofu making are also available (Chang and Hou 2004; Chang 2006). Soymilk is an intermediate product for tofu making. The technology for making soymilk for tofu making is similar to that for making the unflavored plain soymilk products (Chen 1989). This chapter will focus on the basics in the making plain soymilk, which can be used as the base for flavored soymilk products, and on tofu and the factors affecting tofu production and quality from a different approach.

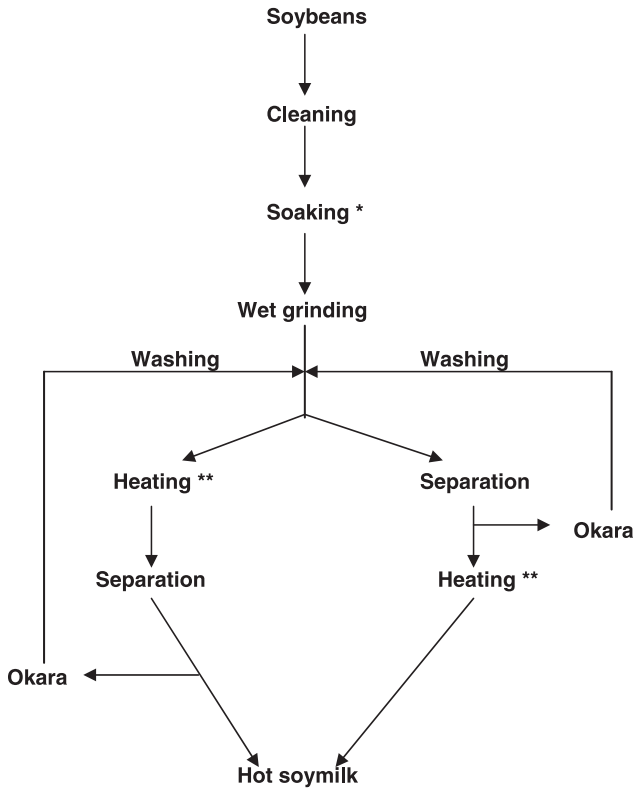

Figure 95.1 *Preparation of soymilk.*

95.2 THE MANUFACTURE OF SOYMILK AND TOFU

There are many methods for producing soymilk (Chen 1989). There are several types of tofu produced nowadays. They are momen (firm or extra firm) tofu, soft tofu, silken (Kinugoshi) tofu, and fill-packed silken tofu. These tofu products are usually packed in trays with water, pasteurized and kept refrigerated in display chambers in retail stores for sell in developed countries. They have a shelf-life of about 3 weeks under proper refrigeration. However, in some countries, they may be sold as fresh tofu in plastic bags or containers with water, or even just cut into smaller blocks as ordered by the consumers. These types of tofu have a short shelf-life, 1–3 days under proper refrigeration. Filled silken tofu has been sterilized and is shelf-stable for 6 months or longer under refrigeration.

Figure 95.1 presents a generalized flowchart in the preparation of soymilk and Figures 95.2–95.4 are schematic diagrams on the manufacture of various types of tofu from soymilk.

95.2.1 Preparation of Soymilk

95.2.1.1 Cleaning and Soaking. Raw soybeans are first cleaned to remove all the foreign matters, followed by soaking the cleaned beans in water to allow the dry beans to absorb enough water before grinding. Soaking time is dependent upon soaking

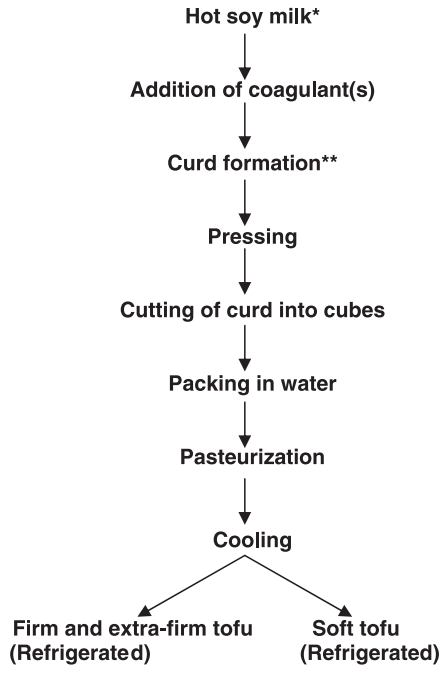

Figure 95.2 *Manufacture of momen tofu.*

temperature. Usually this soaking process takes 8–10 h at 15–20°C or 12–16 h at 10–15°C. This difference is due to ambient temperature difference at various locations of the manufacturing plant. On the other hand, soaking could be done at a low temperature

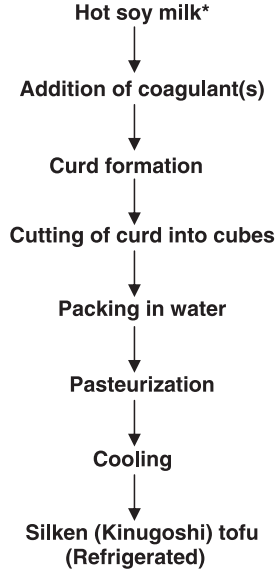

Figure 95.3 *Manufacture of silken (Kinugoshi) tofu.*

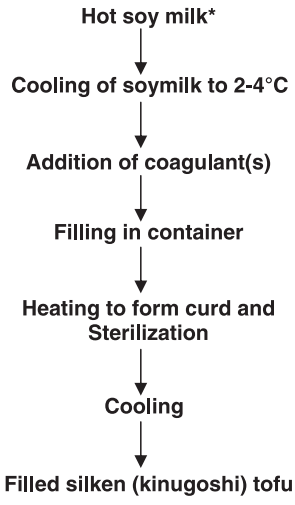

Figure 95.4 *Manufacture of filled silken tofu.*

(2–4°C) to prevent off-flavor development during soaking and grinding. After soaking, the beans weigh approximately 2.2–2.3 times their initial weight. When water is absorbed, it rehydrates the various components in the dry beans making it easy to be dissolved or dispersed in water during the grinding process. Soymilk for tofu making also could be made from soybean powder or soybean flakes (Moizuddin and others 1999) that require less time to rehydrate. In addition, dry unsoaked soybeans or dehulled soybeans can be partially soaked at high temperature for a very short time and milled with hot water (80°C) to extract soymilk with a low beany flavor (Wilkens and others 1967). However, such practice will reduce the yield of soymilk. In preparing consumer soymilk products by some manufacturers, dehulling soybeans prior to extraction also removes germs (hypocotyls), and is carried out to improve the flavor of soymilk and to extend shelf-life.

95.2.1.2 Grinding. Grinding of soaked soybeans breaks down the intact structure of the dry beans. The amount of water added in the grinding process should be carefully controlled to meet the requirements for the different type of tofu to be manufactured. For example, the water dosage for silken tofu, soft tofu and regular tofu is 5, 7–8, and 10 times of raw soybean weight, respectively (Watanabe 1997). This will give soymilk with 9–13 °Brix or higher (see Figs. 95.2–95.4). Grinding can be conducted using a stone mill or stainless steel grinder. Solids content in °Brix can be rapidly measured using a hand-held refractometer for quality control. °Brix is proportionally related to soluble solid content of the soymilk.

Water soluble components such as sugars, amino acids, and water soluble vitamins dissolved in the water added during the grinding process. Proteins and lipids are dispersed in the slurry and the fibers are broken down to smaller particulates. Proper grinding gives appropriate small particle sizes in the slurry and facilitates the extraction of solids and nutrients into the soymilk. It is understandable that the finer the slurry, the more components can be extracted, but it makes it harder for the separation of the soymilk and the residue (*okara*). This will also affect the yield of soymilk.

95.2.1.3 Grinding Temperature. Temperature during grinding affects not only the flavor of the soymilk, but also texture of the resulting tofu. Obata and Matsuura (1993) reported that tofu firmness decreases as the grinding water temperature increases between 0°C and 50°C. They found that this is related to the contents of sulfhydryl (—SH) groups in soymilk that is critical in the curd formation process. Lipoxygenases in soybean decrease the availability of —SH groups in soymilk and makes the tofu less firm.

95.2.1.4 Water Added During Grinding. Tofu contains about 88–90% water. It has long been claimed that water from certain sources such as mountains and streams are better for making tofu. Water containing proper amount (about 100 mg/L) and balance of minerals, including calcium, magnesium, sodium, potassium, iron, and manganese provides a harmonious and mellow taste (Watanabe 1997). The amount of water absorbed during soaking and the amount added during grinding constitute the water-to-bean ratio. For regular tofu, a ratio of 10 : 1 is considered the best with recovered soymilk having 6.0–6.3% solids and 3.0% protein (Watanabe and others 1964). However, ratios of 5 : 1 to 7 : 1 are required for making soft or silken tofu (Saio 1979). For making filled tofu, soy isolate can be added to increase protein content thereby increasing firmness of tofu since pressing is not carried out in filled tofu.

95.2.1.5 Heating and Separation. Heating of the slurry or after separation of the soymilk and the residue serves several purposes:

- Inactivation of microorganisms;
- Improvement of nutritional quality of soymilk by inactivating the trypsin inhibitor (TI);
- Denaturation of proteins to facilitate their curdling in the presence of coagulant(s);
- Improvement on yield of soymilk;
- Reduction of beany flavor.

Abundant of microorganisms are present during the soaking process and introduced during grinding. Mild heating of the soymilk serves as a mechanism to reduce its microbial load, even though it will not destroy all the microorganisms at this stage.

One of the major disadvantages of soybeans is the natural presence of trypsin inhibitor (TI) that affects the digestion of proteins by trypsin. Typsin inhibitor is heat resistant. Upon heating, activity of this trypsin inhibitor can be reduced considerably. Chang and Hou (2004) summarized the effect of various combination of temperature and time of heating to reduce the TI activity. Watanabe (1997) recommends that heating soymilk for 3–5 min at 100°C is adequate for tofu making. Heating temperature and time range of 80–105°C for 4–20 min are used by tofu manufacturers. These heat treatments can reduce the TI activity considerably and at the same time facilitates the separation of soymilk and *okara* (residue). UHT processing has been recently used to heat soymilk in a very short time within one min depending upon temperature (135–150°C) (Kwok and others 1993, 2002; Rouhana and others 1996). The optimal residual trypsin inhibitor activity is in the range of 4–10%. However, UHT heating could result in significant trypsin inhibitor activities if not heated properly (Guo and others 1997a; Xu and others 2004). UHT processing of soymilk has been used to produce aseptically packaged tofu products (Alan and others 1999).

Soy proteins in their nature state maintain their globular structure with the hydrophobic regions wrapped inside. Upon heating, soy proteins are denatured, subsequently unfolding the natural molecules with exposure of the hydrophobic groups to the outside. Thus, protein solubility decreases owing to aggregation. The viscosity of soymilk is affected by heating procedure. A two-step heating method (70°C for 5–10 min followed by heating to 90°C for 5–10 min) resulted in a higher viscosity than that heated by one-step heating (90°C for 5–10 min) (Liu and others 2004; Liu and Chang 2004a).

For small quantity of slurry, separation of soymilk and residue (*okara*) can be accomplished by filtering the slurry in a cotton cloth or nylon bag accompanied by manual pressing. For large volume, it is accomplished by drum pressing, screw pressing, centrifugation, or shake filtration.

The Chinese procedure in the preparation of milk is to separate the soymilk from the residue (*okara*) first followed by heating the soymilk subsequently. However, the Japanese procedure is to heat the slurry first before separation of soymilk and *okara*. It is claimed that the Japanese procedure can increase the soymilk yield and reduce beany flavor. The *okara* contains 17% and 29% of the original protein and solids in the original soybeans, respectively. When the *okara* is rewashed and repressed, additonal 15–20% soymilk can be recovered (Shurtleff and Aoyagi 1990).

Soy products including tofu usually carry a beany flavor that is not familiar to the Western consumers and is a barrier for consumption. The presence of lipoxygenases in soybeans is the major cause of beany flavor in soy products including tofu. The iron-containing lipoxygenases catalyze the oxidation of polyunsaturated fatty acids such as linoleic acid, producing fatty acid hydroperoxides which are then broken to produce hexanal, the major compound of beany flavor in soy products.

Soybeans are known to be the most abundant source of lipoxygenases, with four isozymes, L-1, L-2, L-3, and L-4 (Axelrod and others 1981). L-3 and L-4 are very similar in behavior and composition and they are sometime considered as one isozyme, L-3. Heating is known to be capable of inactivating enzymes in the raw soymilk. L-1 is heat stable, with loss of activity of 50% at 69°C for 25 min (Christopher and others 1970), whereas L-2 and L-3 are much less heat stable with loss of activity of 50% at the same temperature for 0.7 min (Christopher and others 1970). L-1 has optimal activity at pH 9 and L-2 and L-3 at pH 7. L-2 and L-3 have more activity on fatty acids and triglycerides with increased activity at the presence of calcium ions. However, L-1 is more active on fatty acids, but not activated by calcium ions. Even though L-2 is the least abundant but has the highest specific activity at the positions of 9 and 13 of linoleic acid and is mainly responsible for the beany flavor (Christopher and Axelrod 1971; Christopher and others 1972; Takamura and others 1991).

The beany flavor can be developed rapidly when the substrate is available and the temperature is appropriate. For soymilk, it is at the grinding step where the enzyme, fatty acids and triglycerides are liberated with excess of water present. Wilkens and others (1967) found that when the grinding temperature is at 80°C, no volatiles are formed. Several techniques are developed to overcome this beany flavor by heating the soybeans mildly, adjusting the moisture or pH, or using aqueous alcohol to soak the soybeans or their combinations (Hajika and others 1995; Trawatha and others 1995). Soybean cultivars with the absence of lipoxygeanses are also available (Wilson 1996). Soymilk

made from lipoxygenase-free cultivars has the same functional properties, less beany flavor and less astringency as compared to soybeans made from regular soybeans, but the soymilk is rated more yellow and darker (Torres-Penaranda and others 1998). Fatty acids also play an important role in generating beany flavor compounds in addition to lipoxygenases (Yuan and Chang 2007a). Hexanal can be produced by lipoxygenases-null cultivars by autoxidation of unsaturated fatty acids, particularly the linoleic acids (Yuan and Chang 2005a,b, 2007a).

Besides contributing to the beany flavor in soymilk, lipoxygenases have also been found to affect tofu texture (Obata and Matsuura 1993; Obata and others 1996). During grinding of soaked soybean at 2–50°C, lipoxygenases not only oxidize lipids to hydroperoxides, but also subsequently oxidize the free –SH groups liberated from the denatured proteins to disulfide bonds, and possibly cysteinic or cysteic acids. Oxidation of the –SH groups affects their availability to participate in the interchange of free –SH groups with disulfide bonds during heating to form the protein networks, thereby decreasing the firmness of the tofu products. Firmer tofu products can be prepared by grinding soybeans under anaeorbic conditions. Among the lipoxygenase isozymes, L-2 has the greatest SH-degrading capacity.

95.2.2 Coagulation of Soymilk

Coagulation of soymilk to form the curd is the most critical and difficult step in tofu manufacturing as there are many interdependant variables that can affect the outcome of coagulation. These are

- Solid content and pH of soymilk;
- Types and amount of coagulant(s) used;
- Temperature;
- Coagulation method;
- Cutting or breaking curd; and
- Pressing.

95.2.2.1 Solids Content and pH of Soymilk. Soymilk for tofu manufacturing has solid concentration ranging between 9–13 °Brix. For regular or firm (*momen*) tofu manufacturing, it is between 9–10 °Brix. For making silken (Kinugoshi) and filled silken tofu, it is 13° or higher, and for making soft tofu, it is 10–12°Brix. In the making of regular or firm tofu, even though it requires a lower solid content in the soymilk, it takes longer time to coagulate the curd and thus more solids can be coagulated. In addition, it requires pressing to firm the tofu. For making soft tofu, a higher solid content requires shorter coagulation time as compared to the regular or firm tofu. For silken tofu, the soymilk is coagulated without the excretion of whey and there is no pressing, the requirement for a higher solid/protein content in the soymilk is understandable. Soy protein isolate has been added to increase protein content in making filled tofu products. The pH of soymilk before coagulation usually is about neutral or slightly below as this is affected by the original pH of soaking and water added during grinding. This near neutral pH in soymilk is therefore most suitable for lipoxygenases to work on the substrate to produce the bean flavor compounds as indicated earlier.

95.2.2.2 Types of Coagulants Available. Hot soymilk is usually coagulated to form curd by the addition of a salt, and/or acid coagulant. Tofu coagulants are classified into four basic types:

- Chloride-type or *nigari*-type coagulant such as calcium chloride, magnesium chloride, and seawater;
- Sulfate-type such as calcium sulfate, and magnesium sulfate;
- Gluco-delta-lactone (GDL);
- Acid coagulants such as citrus juices, vingar, and lactic acid.

Each type of coagulant has its advantages and disadvantages. *Nigari* may be natural, or refined. Regular *nagari* is composed of mainly magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, 92.3%) and the residual components in seawater (magnesium sulfate, 3.8%; sodium chloride, 1.7%; and calcium sulfate, 1%) after salt (sodium chloride) is removed and dried. Refined *nagari* is mainly magnesium chloride (99.5%). Calcium chloride is not found in seawater but it gives tofu an excellent flavor, almost identical to tofu made with regular or refined *nigari*. In addition, it is generally recognized as safe (GRAS) in the United States, available in food-grade and the cheapest chloride-type coagulant. Tofu made with *nigari*-type coagulant is considered the most delicious with subtle sweet flavor and aroma. However, compared with tofu made with calcium sulfate or GDL, they react very rapidly with soymilk, and therefore require skill and attention. They must be added slowly, and the curd is destroyed while agitation is going on. This type of tofu contains less water as compared to the sulfate-type, and the yield is less with a coarser texture. *Nigari* is not suitable for making silken tofu as the reaction is too fast for the high solid content and high temperature of the soymilk, making it very difficult to form uniform blocks of tofu. However, for filled silken tofu, this problem can be overcome by cooling down the soymilk to 2–4°C prior to coagulant addition followed by heating to slowly coagulate the proteins.

Calcium sulfate or gypsum is the most widely used sulfate-type coagulant in making of tofu in the world. Gypsum is not that water-soluble at room temperature (3.0 g/L), making it a determining factor on the coagulation reaction. It reacts slowly with the soymilk; consequently it allows formation of curds with higher water-holding capacity and soft and smooth texture. Thus, they give 15–20% higher bulk yield as compared to the *nigari* process. Calcium sulfate can be used in the making of regular, firm, soft, silken and even packed (packaged-filled) tofu, whereas as the last two types can not be easily accomplished with *nigari.* Calcium sulfate is easy to use, even with unskillful tofu manufacturers with fairly consistent yields and texture. Calcium sulfate-coagulated tofu has a mild or bland taste and the taste is slightly inferior to that of *nigari*-tofu. It should be noted that coagulation of soymilk using salts followed by pressing will remove the water soluble constituents not trapped in the final curd.

Glucose-delta-lactone (GDL) was first used in Japan to make silken tofu in the 1960s (Shurtleff and Aoyagi 1990). It differs from the chloride- and sulfate-type coagulated tofu in that it uses an acid to form the curd. In a water solution, GDL is slowly hydrolyzed into gluconic acid by water in 2–3 h with a pH drop from 3.5 to 2.5 for a 1% solution at room temperature. In the manufacturing of packed and silken tofu, GDL is dissolved in previously cooled soymilk to allow formation of gluconic acid gradually. Upon heating, the soymilk coagulates due to the action of gluconic acid with the formation of a

homogenous solidified curd without loosing any of the water in the container. GDL-coagulated tofu has a sour flavor and its sourness increases with the increasing concentrations used in making firm and extra firm GDL-tofu. GDL is often used together with calcium sulfate to improve the flavor of the final tofu product. This type of tofu contains all the components of the soymilk used to make tofu in addition to the coagulant.

The acid-type coagulant such as vinegar, lactic acid, and lemon juice can be used as natural coagulants. However, use of this group of coagulant produces low yield with slightly crumbly texture and a slightly tart flavor as compared with the *nigari*- and sulfate-type tofu.

95.2.2.3 Amount of Coagulant Used. The amount of coagulant required to reach the optimum coagulation varies with the solid content of the soymilk. It is also kind of proprietary to the tofu manufacturers. It greatly affects the yield, texture, taste, and aroma of the final product. In general, tofu makers can tell by observing the curd formed and whey produced during pressing whether appropriate amount of coagulant has been added. Whey usually is transparent and amber or pale yellow. When proper coagulation is achieved, the curds have a smooth and cohesive texture in traditional tofu making. If coagulation is incomplete (too little coagulant), the whey is cloudy containing some uncoagulated soymilk. When too much coagulant is used, the texture will become coarse and crumbly. There are some published methods for determining appropriateness of coagulation. Whey transmittance is one of the more reliable guide in determining the optimum amount of coagulant added for maximum yield. Watanabe and others (1964) reported that more concentrated soymilk requires 3–8% more coagulant to reach the same level of whey transmittance, and the dosage for calcium sulfate is 20% than that for *nigari*. Chang and Hou (2004) reported that the coagulant concentration required for reaching optimum coagulation of silken tofu increases linearly with the soymilk solid content in the range of 6–11%, and the concentration for magnesium chloride is about 13–15% more than that for calcium chloride. Kao and others (2003) reported in making firm tofu by calcium sulfate, 0.4% resulted in the most uniform and homogeneous microstructure and retained the highest protein and water in the tofu gel. Whey conductance is claimed to be a better indication for proper coagulation with faster and more reproducible results than whey transmittance (Beddows and Wong 1987a). A rapid titration method based on the flow properties of the soymilk during titration with coagulant was developed in our laboratory (Liu and Chang 2003a,b; 2004a,b) for determining optimal coagulants of the *nigari* type (calcium chloride and magnesium chloride). This method allows the manufacturer to determine coagulants rapidly for reducing waste that may be caused by improper use of *nigari* coagulants. The procedure can be modified for sulfate type of coagulants.

95.2.2.4 Coagulation Temperature. Coagulation temperature is another crucial factor in tofu making. The rate of soymilk coagulation and tofu quality are affected by the soymilk temperature. The yield and moisture content of tofu decrease as the temperature of coagulation increases, whereas hardness and elasticity of the resultant tofu increase (Wang and Hesseltine 1982). When soymilk is at high temperature, proteins possess high active energy. This can lead to fast coagulation, resulting in the formation of curd with low water holding capacity; consequently, tofu has hard texture and low bulk yield. The hotter the soymilk at the time of coagulation, the less the amount of coagulant required. When tofu is coagulated at a high temperature, a small increase in

the amount of coagulant may lead to a large decrease in yield. In the tofu industry, the temperature of coagulation varies from one factory to another, depending on the type of coagulant used. Generally, coagulation temperature ranges from 68°C to 95°C for those using *nigari*, while those using calcium sulfate prefer the range from 70°C to 80°C in Japan. Beddows and Wong (1987b) reported that the optimum coagulation temperature is 75–80°C for silken tofu with gypsum as coagulant in a small bench scale. Shih and others (1997) reported that the optimum coagulation temperature is 85–91°C for making soft tofu with $CaSO_4$ in a medium scale. The operational temperature of coagulation also varies from one region to another. In America, tofu makers prefer a relatively high temperature, 85°C, for using *nigari* and calcium sulfate, since less coagulant is required, the curd forms quickly, and the tofu has firm and dense texture but no significant drop in yield.

95.2.2.5 Coagulation Method. The coagulant addition procedure, the stirring speed, and the follow-up stirring after the coagulant is added have very definite effect on tofu yield and quality. Generally, the controlling techniques of this coagulation rely on the experienced tofu maker's judgement.

Traditionally, calcium sulfate in a suspension is added to soymilk, which has been stirred vigorously by hand with a paddle, and the mixture continues to be mixed six to eight more times.

Nigari-type coagulant could be divided to three portions and added in three steps in order to coagulate soymilk slowly and get high yield and smooth texture. The first portion of *nigari* is poured from a height of several feet into the soymilk being swirled with a paddle. Coagulation starts from the bottom of the container and slowly works up, while the uncoagulated soymilk constantly rises to the surface. The second portion is sprinkled over the soymilk surface. The content is covered and stands for about 5 min. Then, the last portion of the *nigari* is sprinkled over the surface. The curd is allowed to stand for 15–20 min to solidify completely (Shurtleff and Aoyagi 1990).

Optimum combination of stirring time and speed are critical in obtaining quality tofu with proper yield. This depends considerably on the experience of the tofu manufacturer and his desired yield and quality. Chang and Hou (2004) and Shih and others (1997) determined the optimum combinations of soymilk solids, coagulant concentration, soymilk temperature for adding coagulant, and stirring time after adding coagulant for soft tofu making by using a medium scale and a stirrer fixed at 285 rpm. Tofu yield is affected mainly by soymilk solid content and coagulant concentration. Tofu solids and protein content are affected by soymilk solids, coagulant concentration, and stirring time. Solid content of soymilk is the most important factor affecting textural properties of tofu. The optimum combinations are soymilk 11.8–12.3 °Brix, coagulant 0.27–0.32% of soymilk volume, stirring temperature 85–91°C, and stirring time 5–11.3 s (Shih and others 1997).

95.2.2.6 Cutting of Curd. Depending on the type of tofu manufactured, the curd may or may not be cut or break up before pressing to expel the whey. Cutting or breaking curd is required in the manufacturing of regular or firm and extra firm tofu, whereas filled silken and aseptically packaged tofu do not require cutting as they are the curd is formed inside the container.

95.2.3 Pressing

For tofu requiring a cutting step, a pressing step follows to expel the whey. The yield, moisture contents and texture in these final products therefore depend on the pressure applied during this pressing step and its length. It is obvious that longer time and higher pressure are needed for firmer tofu. Pressing usually is conducted in molds of various sizes depending on the volume of tofu to be produced. For research purposes, a mold for small-scale production has dimension of 12.5 L × 12.5 W × 5.5 H cm; a mold for medium-scale production has dimension of 25 L × 25 W × 7 H cm and a mold for large-scale production has dimension of 40 L × 40 W × 4.5 H cm. For industrial production, the molds are much larger. Widely inconsistent conditions of pressures and duration of pressing have been used by different researchers (Chang and Hou 2004) for making various types of tofu. Because of differences in soymilk and tofu making methodology, it is difficult to compare reported data and a standardized procedure is needed for determining the quality of soybeans in tofu making. As a general guideline, tofu manufacturers apply a light initial pressure of 2–4 g/cm^2 for about 5–10 min followed by a stronger pressure of about 5–15 g/cm^2 for 10–15 min to make soft tofu. For firm tofu, a pressure of 20–100 g/cm^2 is used for 20–30 min (Shutleff and Aoyagi 1990). Generally, silken tofu is not pressed, but Beddows and Wong (1987b) reported an optimum pressure of 4–6 g/cm^2 for pressing silken tofu until dripping ceased. Pressures above or below this range will produce unacceptable silken tofu.

95.3 EFFECT OF SOYBEANS AND COMPONENT ON SOYMILK AND TOFU MANUFACTURING AND QUALITY

95.3.1 Soybean Raw Materials

Soybean cultivar is one of the major factors influencing the quality of tofu. It is well known that good tofu can only be prepared from good soybeans. In addition, location (environment of growth) and handling practice at harvest, and storage practices postharvest together can also affect soybean chemical compositions, which in turn affect curd formation and sensory properties of tofu.

Over the years, substantial interest has been placed on the understanding of the quality of various soybean cultivars for tofu making. This is of practical importance in soybean trading since a good quality identity-preserved soybean cultivar developed especially for tofu making would commend a premium. Several researchers have reported the differences in the quality of various soybean cultivars for making tofu. Soybean cultivars with higher protein content have generally lower oil and total sugar content. The chemical composition of soybeans is closely related to that in soymilk and tofu (Wang and others 1983; Lim and others 1990). The higher protein content in soybeans, the higher protein content is retained in soymilk or in packed tofu. However, cultivars with higher protein contents may not produce tofu with harder texture, because protein content alone is not adequate to explain the observations related to hardness. Therefore, a thorough understanding of the composition in various cultivars and changes during storage is important to relate to tofu quality.

Unfortunately, there is no standard method to evaluate soybean quality for making tofu. It is important that an evaluation method using small scale production has the ability to detect the differences of soybeans with different quality characteristics, and such a method could produce a similar trend of results in a large-scale tofu manufacture

process. Since manufacturing processes of tofu vary from manufacturer to manufacturer, one evaluation method can not be applied to all manufacturers. However, for a simple purpose of comparison among different varieties/materials for tofu making, one method developed for a specific tofu product may be appropriate. Most methodologies reported for tofu making are small-scale methods and have not been described in details and they have not compared the reported small-scale method with a large-scale method. Our research results (Cai and others 1997; Chang and Hou 2004) revealed that the small bench and the large-scale method correlated significantly ($P > 0.05$) in tofu yield, color, texture, and chemical composition (moisture, protein, lipid, ash, calcium, and magnesium). The quality and yield of tofu were significantly affected by soybean cultivars and processing methods. Since tofu quality made by the small-bench scale was well correlated to the production method, the bench scale method may be used for determining the quality of soybeans for making tofu. Murphy and others (1997) also suggested a production-scale method for determining the soybean quality for suitability of commercial processing. However, the small bench-scale method developed in our laboratory is appropriate for evaluating soybean quality, since tofu quality made by the small-bench scale is well correlated to a production method.

95.3.2 Storage Conditions

Most soybeans are produced by a few major countries and shipped to other nonproducing countries for further processing into various soy products including tofu (Chang and others 2004; Hou and Chang 2005a). These beans may be stored up to one year or longer after harvest in a wide variety of environmental circumstances before they are processed. Prolonged postharvest storage of beans, generally called aging will decrease the quality of edible soybeans and the viability. The mechanism of soybean aging is not completely understood. A commonly acceptable hypothesis is that lipid peroxidation plays an important role in the initial stage of seed aging process (Parrish and Leopold 1978; Stewart and Bewley 1980; Wilson and McDonald 1986). Hydroperoxides resulted from lipid peroxidation of polyunsaturated fatty acids in the presence of oxygen not only destroy the lipid itself, but also damage cell membranes and other cellular components. In addition, they can break down to form secondary volatile oxidation products, which may contribute to the off-flavor formation in soy products during storage of soybean (Clark and Snyder 1991; Hou and Chang 1998). The magnitude of the quality deterioration of seeds depends upon storage conditions, including time, temperature, relative humidity (RH), and microbial contamination. Among these factors, relative humidity/water activity is the most important. Low humidity may effectively preserve the original bean qualities even at a high temperature (Saio and others 1980). In general, when the relative humidity increased to 80% and temperature is 30°C or higher, the soybean quality deteriorated quickly. Humidity has a higher impact on the soybean quality for soymilk or tofu making, whereas when humidity is low, temperature increase is important in soybean deterioration.

The reported changes of components in soybeans induced by storage include the following:

- Surface discoloration, Maillard reaction, loss in protein extractability (Narayan and others 1988; Thomas and others 1989; Chang and others 2004; Kong and others 2004; Hou and Chang 2005a; Kong and Chang 2006);

- Increase in the acidity or decrease in pH (Hou and Chang 1998; Chang and others 2004);
- Decrease in phospholipid content (Nakayama and others 1981; Mounts and Nash 1990; List and Mounts 1993);
- Changes in isoflavone forms with a decrease in malonyl forms and increase in aglycone forms (Hou and Chang 2002);
- Decrease in extractability of glycinin and β-conglycinin, and changes in the subunit-composition of glycinin and structures (Murphy and others 1997; Saio and others 1982; Chang and others 2004; Hou and Chang 2004; 2005b).

When soybeans are stored in adverse conditions, decrease in soymilk quality, and the yield and quality of tofu with off-flavor and a coarser texture are the results (Hou and Chang 1998, 2005a; Genovese and Lajolo 1992). However, soybeans in conditions of 57% RH 20°C, cool 4°C, or in an uncontrolled ambient temperature condition in North Dakota could remain their soymilk and tofu qualities for up to 18 months (Hou and Chang 2005a,b). In general, whole soybeans are more resistant to deterioration during storage than soy meal or damaged beans including split and seedcoat cracking. Usually, the amount of broken or damaged beans tends to increase with prolonged storage, especially when moisture content is low (<13%) (Genovese and Lajolo 1992).

Under the conditions of 30°C, 82% RH, reducing sugar content in soybeans was first reduced and later nonreducing oligosaccharides in the soybeans were hydrolyzed. A part of the reducing sugars formed by hydrolysis of the oligosaccharides may participate in the nonenzymatic glycosylation and in the Maillard reactions with the amino residues in the soy proteins (Wettlaufer and Leopold 1991; Locher and Bucheli 1998; Hou and Chang 2004, 2005a,b). Sugar content in soybeans and tofu has significance in color and sweetness of the products.

Phytic acid affects the coagulation of soymilk during tofu making by decreasing pH after calcium salt is added. Phytate in soybeans degrades gradually with storage time in the adverse environment. However, under the mild or cold conditions, hydrolysis of phytate also could occur, but at a lesser degree (Hou and Chang 2003). The hydrolysis of phytate in soybeans during storage contributes not only to the decrease of soymilk pH, but also to the loss in chelating ability with calcium ions, and subsequently affects protein coagulation behavior to lead to softer textural quality (Hou and Chang 2003). The effect of phytate on soymilk and tofu making and quality is discussed in the following Section 95.3.5.

95.3.3 Proteins

On a dry basis, about 35–40% of soybeans is made up of soy proteins (mostly globulins) and approximately 90% of these proteins is extractable with water or dilute salt solutions. Soy proteins consist of discrete groups of polypeptides that have a wide range of molecular size. A typical ultracentrifuge pattern of water-extractable soy proteins has four major fractions designated as 2S, 7S, 11S, and 15S on the basis of their sedimentation rates. Each fraction is a complex mixture of proteins. The 7S and 11S proteins are the two major storage proteins in soybeans, which comprise approximately 70% of storage protein. The 2S fraction accounts for about 20% of the extractable proteins, which

contain protease inhibitors (the Kunitz and the Bowman-Birk trypsin inhibitors) and cytochrome C (Steiner and Frattali 1969).

The 7S fraction has been classified into three major components with different physicochemical properties named β-conglycinin, γ-conglycinin, and basic 7S globulin (Catsimpoolas 1969; Hirano and others 1987). β-conglycinin is the most prevalent of these three and accounts for about 30–35% of the total seed protein, which is used interchangeably with 7S protein since it is the major 7S protein. The 11S fraction, designated as glycinin, accounts for an additional third of the total seed protein and is generally simple protein. The 15S fraction accounts for approximately 10% of the total seed protein, which is an aggregate of 11S protein (Wolf and Nelsen 1996).

Tofu is made from heated soymilk that is a turbid solution containing approximately 5% protein and 3% lipid. Soy proteins are the dominant components in tofu dry matter (more than 50% of the total solids on dry basis), which provide the major network structure of tofu gel. Soy proteins form an emulsion gel by a combination of heating and the addition of a coagulant, which is either an acid or divalent salt or a combination of both. This tofu emulsion is permanent since heating is not able to separate lipids from the protein system. Besides protein and lipid, other components in soymilk such as phytate, isoflavones, saponins, and lipoxygenases also may play important roles in coagulation of proteins during curd formation. Because of the complexity of the soymilk-tofu system, the mechanisms of tofu formation are also complex, and are not fully understood. Even though reports on understanding the interactions between nonprotein constituents (e.g., phytate and lipid) and proteins on the coagulating reaction in tofu making are available (Saio and others 1969; Kumagai and others 1998), there is a need for a comprehensive approach to put all factors together in one picture to understand the gel formation in tofu making.

Recent studies have shown that various soybean cultivars have various ratios of 11S/7S proteins that may influence the textural quality of tofu (Cai and Chang 1999; Poysa and Woodrow 2002; Mujoo and others 2003). We found that the β-conglycinin (7S) and glycinin (11S) contents in 13 varieties are 17.2–23.1% and 36.3–51.3% of total proteins, respectively, and the 11S/7S protein ratio varied from 1.64 to 2.51 among the varieties (Cai and Chang 1999). Furthermore, positive correlations existed between tofu firmness and the 11S/7S ratios in various (13 to 16) soybean cultivars (Zhang and Chang 1996; Chang and Hou 2004; Cai and Chang 1999). Processing methods also affect 7S- and 11S-protein content of tofu and their contribution to tofu hardness, yield and sensory quality. Thus, processing methods have an impact on the relationships between 11S/7S ratios and textural quality since different coagulation processes and pressing steps are used for preparing tofu (Cai and Chang 1999). However, conflicting results on the relationships between 11S/7S ratio and tofu firmness have been reported by other researchers (Murphy and others 1997; Skurray and others 1980; Utsumi and Kinsella 1985; Taira 1990). The conflicting report may be partly due to different methods used for processing, because of a lack of standard methods for tofu research.

95.3.4 Lipids and Phospholipids

Approximately 60% of total lipids in raw soymilk are associated with the protein particles, however, only 3% are found in the protein particles of cooked soymilk (Ono and others 1996). Significant amount of lipids in raw soymilk can be precipitated with large protein particulates by low speed centrifugation (Liu and Chang 2006). After being

heated to 65°C, part of the lipids and almost all α and α′ subunits of β-conglycinin in the particulate fraction begin to liberate to soluble fraction. Above 90°C, almost all neutral lipids in the protein particles of raw soymilk is liberated to a floating fraction, and one half of the phospholipids remains in the particles (Ono and others 1996; Guo and others 1997b). Coagulation of soymilk depends on the concentration of coagulant, the pH of soymilk, and temperature, the last being an external factor that accelerates soymilk coagulation. In fact, the addition of coagulant causes not only protein coagulation and gelation but also the incorporation of lipids into the protein gel with the lipid droplets being located in the networks of the protein gel (Saio and others 1969; Yamano and others 1981; Guo and others 1999).

Phospholipids (including lecithin) are polar compounds. More than 50% of these lipids extracted from hexane-defatted soy meal are phopsholipids. They are believed to play an important role in combining the particulate proteins with neutral lipids (Ono and others 1996). 11S and 7S proteins isolated from defatted soy meal contain about 0.8% and 2.3% phospholipids, respectively. Phospholipids bind to the hydrophobic sites of β-conglycinin (Ohtsuru and others 1979). 7S protein is more hydrophobic than 11S protein and therefore phospholipids bind stronger to the 7S proteins. Removal of lipids, particularly the phospholipids, from the surface of 7S proteins by extraction with chloroform:methanol solution makes 7S proteins vulnerable to form insoluble aggregates thus decreasing the ability to complex with protein particles. Adding phospholipids to soymilk increases the formation of protein particles. Lecithin-supplemented gels exhibit a fine network structure.

95.3.5 Phytic Acid

Phytic acid (phytate) content comprises 1~2% of soybeans on a dry basis and accounts for about 70∼80% of the phosphorus in seeds (Cheryan 1980). It is structurally integrated with the protein bodies as phytin, a mixed potassium, magnesium, and calcium salt of inositol (Erdman 1979). Phytate has strong chelating ability with multivalent metallic ions, especially zinc, calcium, and iron. According to Graf (1983), calcium ion can bind to phytic acid over a wide pH range (pH 4.8–10.4), the degree and tightness of binding being affected by pH, temperature, ionic strength, and size and valence of the cation. The affinity of phytic acid for calcium increases sharply with pH; the higher the pH (alkaline), the higher affinity; the affinity in pH 10.4 is a thousand-fold higher than in pH 4.8.

It is reported that phytate attaches to the glycinin at pH between 2.5 and 5.0, and the extent of binding increases with decreasing pH; above the pI (isoelectric point) of glycinin (pH 4.9), no binding is found (Okubo and others 1976). A portion of phytate in soymilk is bound to particulate and soluble proteins (about 35% and 23%, respectively). The others are present in the free form (about 42%) (Ono and others 1993). Therefore, when calcium is added to soymilk, it binds to phosphate groups of phytate and binds to proteins as well.

The role of phytic acid in the coagulation step during tofu-making has been related to a decrease in pH after calcium salt is added (Ono and others 1993; Tezuka and Ono 1995). When calcium is added, the phytate-calcium salts form at approximately the neutral pH (6.6). Hydrogen ions bound originally with phosphate groups in phytate, are liberated. Therefore, the pH decrease upon the addition of calcium may be mainly due to the formation of phytate-calcium salts. The decrease in pH of soymilk from approximately 6.6 to 5.8 after the addition of calcium chloride allows the use of a lower concentration of Ca salts for coagulation. When calcium is present, it binds simultaneously to proteins

and phytate, calcium binding to proteins can retard the decrease of pH because of less phytate-calcium formation. Tofu curd contains both types of calcium bound to protein or phytate.

Phytate is also very important in relation to the speed of coagulation during tofu making. It has been found that higher content of phytic acid results in a slower coagulation of soymilk during tofu making, and gives a higher tofu yield (Saio and others 1969). Therefore, the phytate content in soymilk can affect the textural properties of tofu. To understand the role of phytate on yield and textural properties of tofu, we conducted a series of experiments using Proto soybean as the raw materials that were stored under a series of environmental conditions to induce the changes of phytate (Hou and Chang 2003). Significant positive relationships exist between soybean phytate and tofu yield ($r = 0.93$) and between soymilk phytate and tofu yield ($r = 0.95$). Negative correlations between soybean phytate and tofu hardness and brittleness are observed ($r = -0.92$ and -0.84, respectively). Negative correlation coefficients are found between soymilk phytate and tofu hardness and brittleness ($r = -0.94$ and -0.86, respectively). Although statistical correlation between phytate content existed, phytate does not have a direct impact on tofu yield since when phytate changes in soybeans during storage, other components such as protein solubility, protein molecular structures, titratable acidity also are changed simultaneously (Hou and Chang 2004, 2005a,b). To understand the separate effect of phytate, phytase was used to hydrolyze the phytate in soymilk to that of the stored beans. Reducing phytate actually increases tofu yield but reduces firmness. Therefore, phytate plays a minor role in affecting tofu quality as long as the coagulant used is optimal. Besides its effect on texture of tofu, phytate has been recently reported to be responsible for the astringency of soymilk (Mahfuz and others 2004a,b). The astringency in soymilk disappears when the soymilk is coagulated with calcium salt to tofu. Therefore, the calcium salt form of phytic acid has no astringent taste.

95.3.6 Isoflavones and Saponins

Distribution of isoflavones, a subclass of the more familiar flavonoids, is extremely limited in nature. Soybeans and soy foods are the major foods containing significant amount of isoflavones. The main isoflavones found in soybeans are genistein, daidzein, and glycitein, each of which exists in four chemical forms, as an aglycone form (genistein, daidzein, and glycitein), a β-glucoside form (genistin, daidzin, and glycitin), a malonylglucoside form (6″-*O*-malonylgenistin, 6″-*O*-malonyldaidzin, and 6″-*O*-malonylglycitin), and an acetylglucoside form (6″-*O*-acetylgenistin, 6″-*O*-acetyldaidzin, and 6″-*O*-acetylglycitin).

Isoflavone content varies among soybean varieties, which contain approximately 1–4 mg/g soybean. The isoflavone content of soybeans is markedly affected by crop year and growing condition (Wang and Murphy 1994; Hoeck and others 2000). Isoflavones are quite heat stable. Wang and Murphy (1994) reported that cooking did not influence the isoflavone retention during tofu making, but alter the distribution of isoflavones by dramatically decreasing in malonylglucoside forms and increasing in acetylglucoside forms. Minimal heat processing can convert substantial amounts of malonylglucoside to the β-glucosides. Total isoflavone content in soy products decreased most likely due to leaching of isoflavones into water during processing. Wang and Murphy (1996) reported that 44% of total isoflavones were lost during the processing of tofu. Coagulant type and concentration affect the recovery of isoflavones in tofu (Kao and others 2004). The concentration of 0.3% calcium sulfate gives the highest tofu recovery as compared to that

made by calcium chloride. Isoflavones have several potential health benefits (Chang and Hou 2004). Liu and others (2005) reported that tofu has *in vitro* and *in vivo* antioxidant activity as high as that of 250 ppm isoflavone crude extracts.

Saponins are widely distributed in plants, and are glycosides composed of a sapogenin that makes up the aglycone and a sugar moiety. The sapogenin is a triterpeneoid alcohol. At least five sapogenins have been found in soybeans (Birk 1969). Xylose, arabinose, galactose, glucose, rhamnose, and glucuronic acid have been found in the glucoside portion of soy saponins. Saponins exist in two groups, A and B (Shiraiwa and others 1991a,b; Kudou and others 1992). The group A saponins consist of 6 different kinds of saponins (Aa, Ab, Ac, Ad, Ae, and Af), which are acetyl-soyasaponins. The group B saponins, on the other hand, consist of eight kinds of saponins (Ba, Bb, Bb′, Bc, Bd, Be, BdA, and BeA), which are not acetylated and are different from the group A. BdA is the major natural soybean saponin in soybean seeds (Kudou and others 1992). Saponins are polar compounds because of the associated sugars (oligosaccharides), which are found in the soybean meal in amounts of approximately 0.5% of the dry weight (Fenwick and Oakenfull 1981).

It is known that isoflavones and saponins impart the bitter and astringent after-tastes in the flavor of soy products. Glucoside forms of saponins and isoflavones are the major compounds that cause objectionable after-taste in soybeans, and saponin A group contributes most strongly to the undesirable taste. The undesirable taste becomes weaker when saponins decompose from glucoside forms to aglycone forms, while isoflavone glucosides show a reverse tendency. The aglycones of isoflavones have stronger objectionable after-taste than those of glucosides (Matsuura and others 1989; Okubo and others 1992).

Saponins, glycitin, and glycitin derivatives are present primarily in hypocotyl, and all of these substances can give objectionable aftertaste. In Japan, removal of these compounds by processing methods has been reported to produce good-taste tofu by the Namashibori technique (Watanabe 1997). In the method, the soybean is first cracked, and seedcoat and hypocotyl are removed. The resulting materials are soaked in water for a short period of time, ground to make the slurry "go" and filtered. The raw soymilk is boiled and the brown foams (which contain saponins) are scooped away to reduce aftertaste. However, in light of potential health benefits of these isoflavone and saponin compounds (Hasler 1998), a different strategy may be needed to preserve these compounds to produce soy foods with the maximum health benefits.

95.3.7 Lipoxygenases

Lipoxygenases and hydroperoxide lyase (Matoba and others 1985) are involved in the oxidation of polyunsaturated fatty acids such as linoleic acid and linolenic acids to hydroperoxides and secondary breakdown products, some of which have beany flavor. Heating can reduce the activity of lipoxygenases and hydroperoxide lyase (see earlier sections on Heating and Separation on reduction of beany flavor, and Grinding Temperature).

95.4 MECHANISM OF TOFU CURD FORMATION

There are several reviews on the mechanism of tofu curd formation (Saio and others 1969; Ono and others 1991, 1993, 1996; Tezuka and Ono 1995; Guo and others 1997a,b, 1999; Ono 2000; Tezuka and others 2000). It could be summarized into three steps.

- The total of the large and medium-sized particles constitutes more than 50% of the proteins in soymilk. Before heating the raw soymilk, most of the particles are still intact. Most lipids are present in the protein particles.
- When the soymilk is heated up to 90°C, the proteins denature and the lipid droplets are liberated to floating fraction. Approximate 75% of the large particles is degraded to supernatant proteins, but the amount of medium-sized particles increases due to the combination of β subunit of 7S and the basic polypeptide of 11S from the supernatant proteins that contain mainly 11S and 7S globulin.
- The addition of coagulant (calcium ion, magnesium ion and/or GDL) to the heated soymilk is the key step. At low concentration of coagulant(s), protein particles combine with lipid droplets and the gel network is first formed through the binding of calcium to protein particles to neutralize the negatively charged protein molecules and to cause protein aggregation due to a reduction in electrostatic repulsion. When about 50% of the proteins is coagulated with coagulant, almost all the lipids in soymilk are trapped and become inseparable by the association with particulate proteins. With the availability of more coagulant(s), it leads to a decrease in pH. The soluble proteins aggregate at a higher concentration of coagulant and bind to the protein particles-oil droplet complex to form a stable tofu-curd emulsion network.

95.5 FOOD QUALITY EVALUATION OF SOYBEANS, SOYMILK AND TOFU: TRADING AND INDUSTRY PERSPECTIVE

95.5.1 Soybean Quality

As discussed above, soymilk and tofu quality characteristics are affected by raw material characteristics, processing and storage. In trading, identity-preserved and organic food soybeans are preferred by the industry. Soybeans are alive and each lot of the soybeans may have unique properties and they can change over time. The seller and purchasers should maintain a record of the history of the soybeans with respect to variety (cultivar), production environment (location, soil, water, temperature, and sunlight of cultivation), harvest and storage conditions (humidity, temperature, and time), and shipping conditions. Mechanical drying after harvest should be done very carefully so that the proteins are not denatured.

The following are the factors that are commonly considered in trading soybeans for food uses.

95.5.1.1 Size and Hilum Color of Soybeans. Traditionally, large size is preferred for making tofu. However, our research shows that size is not a factor to affect tofu quality (Wang and Chang 1995). Size uniformity and % hard-to-soak beans (stone beans) are important. Clear hilum soybeans also have been preferred by the industry. However, our research shows that soymilk and tofu color is not related to hilum color. We have observed that a brown hilum color soybean produces soymilk with whiter color than some white hilum soybeans.

95.5.1.2 Chemical Composition as Related to Food Quality. Protein content and protein subunit patterns (11S and 7S subunit distributions) and lipid content affect the yield, composition, and texture of soymilk and tofu. Lipoxygenases, lipid and fatty acid content affect the flavor of soymilk and tofu. Sugar content affects the taste.

Phytate, saponins, and isoflavones may affect the bitterness and astringency of the soymilk products. However, the exact contributions of these compounds to food quality are not well established.

95.5.1.3 Storage Changes. Undesirable storage changes can be measured. Chemical changes include protein solubility, titratable acidity or pH of soymilk. Rancidity or oxidized off-flavor may be resulted from improper storage. Color is a good indicator of the changes of soybeans during storage and can be measured using a surface colorimeter such as the Minolta colorimeter with the Hunter's Lab scale. The optimal coagulant concentration for making tofu decreases with storage (Liu and Chang 2003a,b). Soymilk and tofu yield and texture need to be measured before trading. Soybeans should be kept in a low relative humidity and cool temperature to preserve the quality for food making (Hou and Chang 2005a).

95.5.2 Soymilk Quality

Indicators for soymilk include protein recovery, sugar content, color, beany aroma or off-flavor, and taste (sweetness, bitterness, astringency, aftertaste, and chalky taste). Instrumental analysis can be used to measure color, whereas chemical contents such as protein recovery and sugar content can be measured. Additional composition of interest could also be measured. Aroma and taste using hedonic or descriptive methods can be used.

95.5.3 Tofu Quality

In addition to yield of tofu such as g tofu made from 100 g soybean, color, texture, (firmness, cohesiveness, elasticity, and smoothness) and taste are important characteristics of tofu. Small-scale (Chang and Hou 2004) or pilot-scale making equipment (Figs. 95.5 and

Figure 95.5 *Tofu machine.*

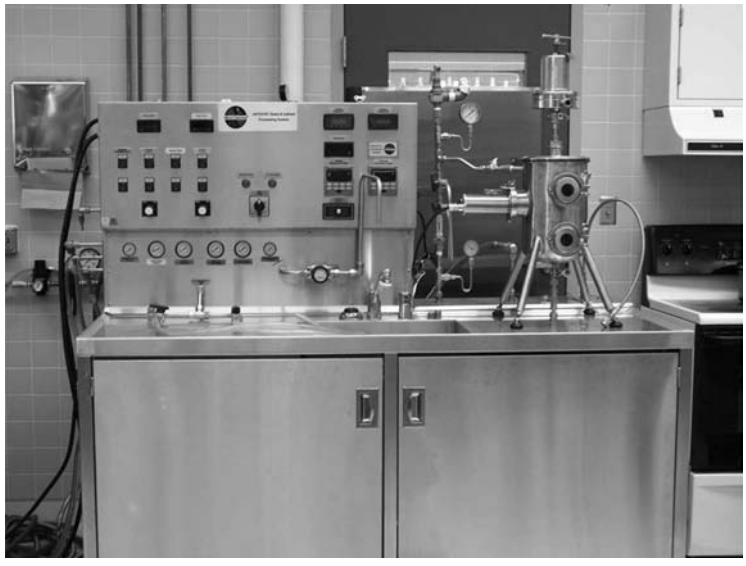

Figure 95.6 *UHT unit.*

95.6) are useful for processing tofu and soymilk for quality evaluation of soybean materials (Cai and others 1997; Chang and Hou 2004). Chemical methods and sensory methods are available for measuring the above characteristics. Instrumental methods are commonly used for textural analyses (Chang and Yuan 2005; Yuan and Chang 2007b). Hexanal, a major indicator of grassy, beany flavor compound commonly analyzed by gas chromatography, is strongly affected by raw materials and processing methods (Yuan and Chang 2005a,b, 2007a). Since tofu researchers use different parameters for textural analysis, the interlaboratory results are difficult to compare. The Instron universal testing equipment (Bourne 1982) is the most reported method for textural profile analysis of tofu (Chang and Hou 2004; Yuan and Chang 2007b). Yuan and Chang (2007b) investigated the optimal Instron settings for measuring textural qualities of 13 different types of tofu and found that the instrumental analysis using the selected parameters (75% plunger penetration and crosshead speed of 60–100 mm/min for cylindrical sample size of 44 mm diameter and 1.5 cm height) correlated very well with the results obtained from the human sensory testing methods.

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96

Tomato Processing

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96.1 Introduction 1092
96.1.1 History 1092
96.1.2 Biology 1092
96.1.3 Growth, Harvesting, and Crops 1094
96.1.4 Production Statistics 1094
96.1.5 Nutritional Value 1095
96.2 Canned Tomatoes: Processing Operations 1096
96.2.1 Sorting and Grading 1096
96.2.2 Washing 1097
96.2.3 Sorting 1097
96.2.4 Coring 1097
96.2.5 Peeling 1097
96.2.6 Blanching 1098
96.2.7 Manual Sorting 1098
96.2.8 Filling, Additives, and Containers 1098
96.2.9 Exhausting and Sealing 1098
96.2.10 Thermal Processing (Canning) 1099
96.2.11 Quality Standards 1099
96.3 Diced Tomatoes: Processing Operations 1100
96.3.1 Size Reduction 1100
96.3.2 Filling, Additives, and Containers 1100
96.3.3 Quality Standards 1100
96.4 Tomato Concentrate or Tomato Paste: Processing Operations 1101
96.4.1 Sorting and Grading 1101
96.4.2 Washing 1101
96.4.3 Break 1101

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

96.4.4 Extraction 1102
96.4.5 Deaeration 1102
96.4.6 Homogenization 1102
96.4.7 Paste Concentration 1102
96.4.8 Aseptic Processing 1102
96.4.9 Quality Standards 1103
96.4.9.1 Mexican Quality Standard 1103
96.4.9.2 International Quality Standard 1104
96.5 Tomato Juice: Processing Operations 1104
96.5.1 Break 1104
96.5.2 Extraction 1104
96.5.3 Deaeration 1105
96.5.4 Formulation 1105
96.5.5 Homogenization 1105
96.5.6 Aseptic Processing 1105
96.6 Tomato Catsup: Processing Operations 1105
References 1106

96.1 INTRODUCTION

Fresh tomatoes are preferred over processed ones in Mexico just like in other parts of the world. Nevertheless, in the last few years the demand for tomato products, such as juices, purees, paste, and sauces, has increased due to technological advancement and the tastes and habits of the new generation.

Tomatoes and tomato products are considered to be healthy food for various reasons: they are low in fat and calories, free of cholesterol, and a good source of fiber and proteins. Tomatoes are also rich in vitamins A and C, ß-carotene, lycopene, and potassium. Tomato quality and its processed products are evaluated according to their redness that is due to their lycopene content. Therefore tomato and its products are the main source of lycopene which makes them important contributors of carotenoids to the human diet (Shi and Le Maguer 2000).

96.1.1 History

The tomato is a plant native to the American tropics that reached its greatest importance and development outside its area of origin and the tropics. The United States and Europe have introduced improved crops in the last few years, especially hybrids that have eliminated native crops of inferior quality. The tomato originated in the Andes region (Chile, Colombia, Ecuador, Bolivia, and Peru (Vavilov 1951) where the greatest genetic variability and abundance of wild types are to be found (Chávez 1980). Mexico is considered by the world, though, to be the most important domestication center of tomatoes. The word tomato comes from the Nahuatl word "tomatl." The Spaniards took this vegetable to Europe in 1554 and its commercialization began in the United States in 1835 (Chávez 1980).

96.1.2 Biology

The tomato (*Lycopersicon esculentum*) belongs to the Solanaceae family and is an annual crop. It can be semiperennial in tropical regions (Fig. 96.1) There are diverse crops with

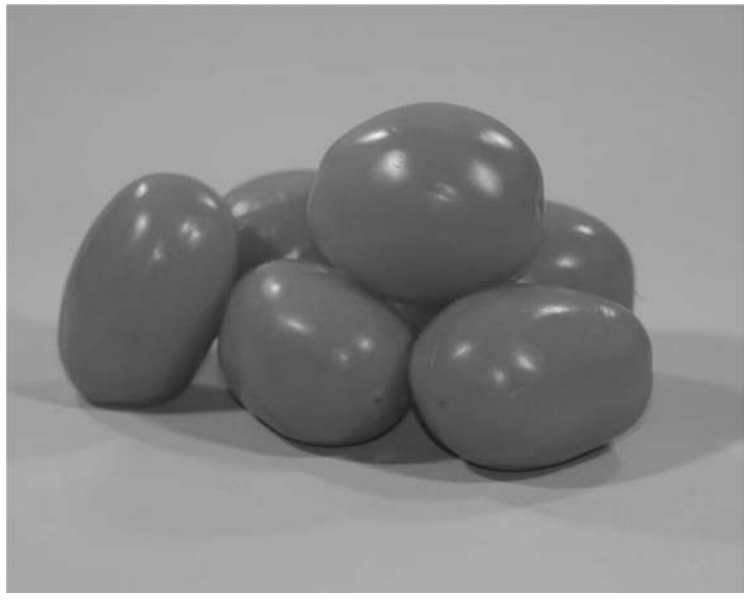

Figure 96.1 *Tomato* (*Lycopersicon esculentum*).

greater yield, resistant to pests and diseases, and that have a more adequate color, viscosity, and solid content. The fruit is a meaty berry composed of various lobules: from two (bilocular) to three or more lobules (multilocular). Commercial crops belong to the locular type (Valadez 1989).

The berry consists of skin over an external wall and internal radial walls of pericarp containing the locular material. The skin is composed of a cuticle over the epidermis. The cuticle contains cuticular acids and waxes that allow the berry to be resistant to attacks by insects and diseases; it also makes peeling by steam or with lye more difficult (Barringer 2004). The red flesh, made up of parenchyma rich in lycopene, is found beneath

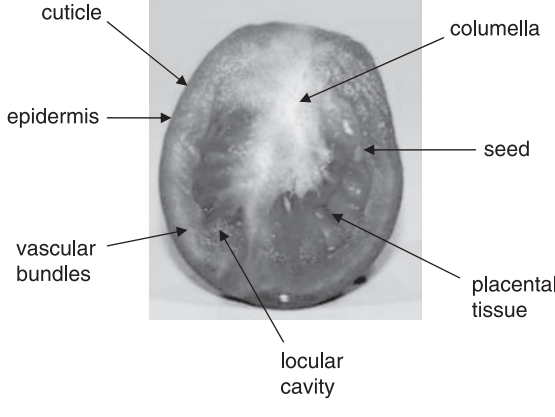

Figure 96.2 *Cross-section of a tomato.*

the skin in the form of crystals suspended in the liquid that fill the parenchyma cells. If the tomato is peeled too deeply, these tissues are lost and the yellow vascular bundles become visible. The seeds are found embedded in the gelatinous parenchyma inside the locular cavities (Fig. 96.2).

96.1.3 Growth, Harvesting, and Crops

The tomato is a warm climate vegetable that is frost intolerant; the temperature range of the soil should be from 12° to 16°C, with a maximum of 30°C, and the air temperature for its development should range from 21° to 24°C, with the optimum temperature being 22°C; growth is stunted at temperatures below 15°C and higher than 35°C. When there are high temperatures (greater than 38°C) for 5–10 days before the anthesis, there is little tying of the fruit because the grains of pollen are destroyed; if high temperatures continue for 1–3 days after anthesis, the embryo is destroyed. The tying of fruit is also low when night temperatures are high (25–27°C) before and after the anthesis. At a temperature of 10°C, or lower, a great percentage of the blossoms drop off (Valadez 1989).

The optimum temperature for fruit maturation is from 18° to 24°C. If the temperature is lower than 13°C, the fruits tend to mature poorly. On the other hand, when the temperature is higher than 32°C during storage, the red coloration (lycopene) is inhibited and the fruit turns yellow. It has been reported that optimum coloration is obtained at temperatures of from 22° to 28°C (Valadez 1989).

The fruit of the tomato is climacteric and may be harvested at different stages of ripening, from its green to red stage. Tomatoes produce small quantities of ethanol during their development and also undergo changes in the presence of exogenous ethylene (Wills and other 1989). Respiration and ripening increase in response to this gas. This last property is used by producers and distributors to accelerate ripening; ethrel is applied commercially.

Tomato crops may be grouped according to consumption type and market; there are markets for it fresh or for industrial production. Crops with lower acidity (pH), higher soluble solids, less viscosity, greater consistency and redness are used industrially (Gould 1992).

Tomato production has evolved in Mexico through the years as a result of the variation that its commercial demand has undergone. Its production has been established outdoors in the ground, in espaliers and lastly, in greenhouses. The genetic progress obtained is so important that new varieties appear each year which displace others that were developed a few years before. Hybrids that retain the most favorable characteristics of the preceding varieties are an important advancement. Some of the varieties grown in Sinaloa and Sonora are Rio Grande, Super Rio Grande, and Big Rio and the tomato hybrids are Saladette: Sauzalito, Brigade, Maya, Yaki, SXT-0289, Tequila, and Santa Fe (Borbón and Armenta 2000). Tomatoes are also classified according to their shape: round or ball, pear or guajillo (Valadez 1989). The round tomato and the saladette, or guajillo, are the most produced, while fewer cherry tomatoes are (ASERCA 1995).

96.1.4 Production Statistics

The tomato is the main export crop in Mexico, said to represent approximately 37% of the value of vegetable exports. Its production extends into 27 states, in the northern, central,

TABLE 96.1 Principal Tomato Producing States in Mexico, 2003.

State	Area Harvested (Ha)	Production (Ton)	Participation (%)
National	67,644	2,171,159	100
Sinaloa	21,908	742,685	34.21
Baja California	5,638	251,057	11.56
Michoacán	6,645	237,454	10.93
San Luis Potosí	6,757	208,798	9.62
Baja California Sur	2,657	122,045	5.62
Other	24,039	609,120	28.06

Source: SIAP (2006).

and southern regions of the country. Even so, approximately 71.94% of the national production is concentrated in five states.

The principal tomato producing states according to production volume are Sinaloa (34.21%), Baja California (11.56%), Michoacan (10.93%), San Luis Potosi (9.62%), and Southern Baja California (5.62%) (Table 96.1; SIAP 2006).

The geographic location of the country and intensive production technology permits exploitation in two known growth cycles: spring-summer, and fall-winter. The latter has the greatest production, for example Sinaloa, with its products sent to national and/or international market, depending on the market demand at harvest time. Production in this state is 61% tomato saladette, 39% round tomato, and 10% industrial (ASERCA 1998).

Sinaloa is the main seasonal exporter of tomatoes because the producers prioritize exports, programming their fields and crops in order to place high quantities of the crop on the market when the prices are high on the border; this situation causes the supply of tomatoes to be deficient in the national market. These market strategies are possible due to the Israeli seeds that are used to produce tomatoes. These seeds require technological packages that involve not only genetically improved seeds, but also drip irrigation and extensive use of greenhouses (ASERCA 1998).

At the world level, the principal tomato producers that produce 60.46% of the total world production (120,384,017 metric tons in 2004) are: China (25.04%), the United States (10.60%), Turkey (6.65%), India (6.31%), Italy (6.23%), and Egypt (5.63%) (Table 96.2, FAOSTAT 2005). Mexico with 1.78% is in tenth place. The principal countries that imported this vegetable in international commerce in 2004 were: the United States, Germany, France, the United Kingdom, and the Russian Federation. The greatest demand for Mexican tomatoes is from the southern and eastern regions of the United States (ASERCA 1998). The principal exporting countries for this same year were Spain, Mexico, the Netherlands, Syria, and Jordan (FAOSTAT 2005).

96.1.5 Nutritional Value

The composition of a tomato depends on the variety, stage of ripeness, climatic growing conditions, light, temperature, soil, fertilization, and irrigation. The water content ranges from 90% to 96%, while its total solids range between 5% and 6% (Table 96.3; Titchenal and Dobbs 2004). Approximately half of these solids are reducing sugars, with a slightly higher amount of fructose than of glucose. Sucrose is generally 0.1%. A quarter of the total

TABLE 96.2 2004 World Production for Tomatoes.

Country	Area Harvested (1000 Ha)	Production (1000 Metric Ton)	Participation (%)
World	4421	120,384	100
China	1255	30,142	25.04
United States	173	12,766	10.60
Turkey	220	8,000	6.65
India	540	7,600	6.31
Italy	135	7,497	6.23
Egypt	191	6,780	5.63
Spain	71	4,366	3.63
Iran	130	4,200	3.49
Brazil	58	3,420	2.84
Mexico	67	2,148	1.78

Source: FAOSTAT (2005).

TABLE 96.3 Tomato Composition Base on 100 g of Edible Material.

Water (g)	Kilocalories	Fat (g)	Protein (g)	Total Carbohydrates (g)	Sugar (g)	Fiber (g)
90–96	14–23	Tr–1.26	0.7–1.2	4.7	1.2–3.4	0.4–1.8

Source: Titchenal and Dobbs (2004).

solids are citric, malic, and dicarboxylic amino acids, lipids, and minerals. The other quarter is made up of proteins, pectic substances, cellulose, and hemicellulose (solids insoluble in alcohol). Tomatoes are an important source of nutrients and vitamins, mainly vitamin A and C (Barringer 2004).

Tomatoes are available all year round in Mexico. A basic crop in the Mexican diet, it is consumed fresh in salads or in food, in processed products such as paste, juice and canned. These products are used to prepare sauces and are eaten in soups, with meat, fish, and chicken. Hot chilli salsas and catsup are used as condiments. Tomato juice has recently increased in popularity.

96.2 CANNED TOMATOES: PROCESSING OPERATIONS

Tomatoes destined for industrialization are harvested at an advanced degree of ripeness, through the use of selected crops with adequate irrigation systems (Fig. 96.3).

96.2.1 Sorting and Grading

Whole canning tomatoes are selected and classified by color, firmness, defects, and size that are evaluated by experts. Red color should be uniform throughout all surface of fruit. Its classification is by color standards which are norms proper to the industry. A Hunter lab, Munsell or Agtron color spectrometer is also used, as well as cameras and infrared light. Firmness and color are parameters for determining the quality of the canned product.

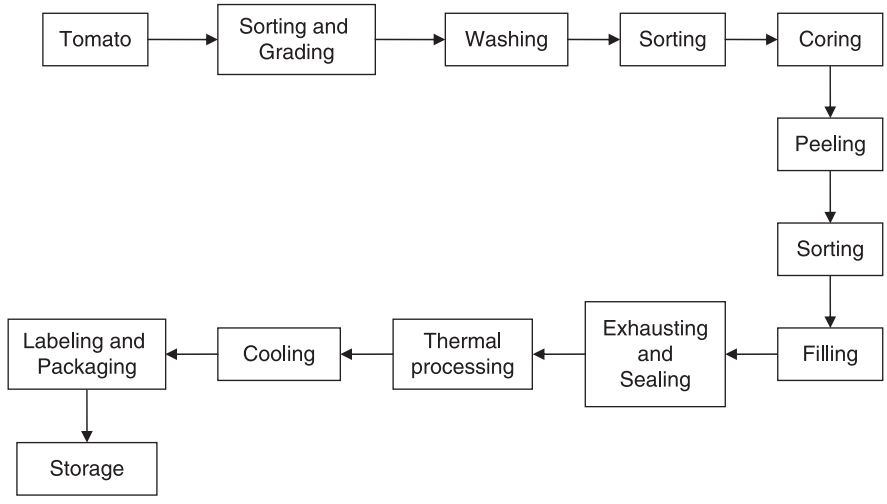

Figure 96.3 *Flow chart of whole canned tomatoes.*

96.2.2 Washing

The tomatoes are washed in agitation tanks for 3–5 min to eliminate dirt and contaminants and to reduce the microbial count. After the dust clinging to the tomatoes has been completely removed, the tomatoes are rinsed under streams of water.

96.2.3 Sorting

Selection can be made according to size, state of maturity, and firmness. Hydrosorter involves an immersion tank that is used to remove the immature fruit during the washing stage. The immature tomatoes float in the hydrosorter tank, while the ripe fruit sinks. The immature product is used for juice. The size sorters eliminate the small tomatoes and send them to the tomato choppers. Sorting according to firmness (texture) eliminates soft ones and sends them to the juice line.

96.2.4 Coring

This operation consists in extracting the peduncle and heart in the section closest to the peduncle. There is machinery that does this, though it can also be performed manually.

96.2.5 Peeling

Peeling is an unavoidable operation in the processing of the majority of fruit and vegetables and involves eliminating the least possible amount of the product. In the case of tomatoes, the peeling methods that are most used in the industry are steam peeling and peeling using lye. In the case of steam peeling, the tomatoes are placed on a moving belt and go through a closed section where steam is injected, and then continue with the next step in the process. Steam peeling is undertaken at 24–27 psi and 127°C for 25–40 s (Gould 1992). The high steam temperature rapidly heats the surface of the

tomato and when there is a sudden fall in pressure (upon exiting the steam chamber), the steam under the skin is released and the superficial cells burst. The majority of the material comes away when the steam is released and streams of water are needed to remove the remaining attached skin. The factors to be controlled during the peeling process are temperature and pressure. Higher temperatures and shorter time yield a more efficient peeling process. The steam peeling method is more commonly used in industry.

Peeling using lye consists of the tomatoes being transported on a belt to an immersion tank of hot lye where they remain for 25–30 s. The concentration is 12–18%, at a temperature of 88–93°C. This chemical peeling process involves chemical and diffusion reactions; the lye dissolves the cuticular wax and hydrolyzes the pectin, reacting with the organic acids in the cytoplasm, middle lamella, and cellular wall, and, as a result, separates the tomato skin (Gould 1992). After either steam or lye peeling, the tomatoes pass through a series of rubber disks or through a rotating drum under high pressure water sprays, to remove the adhering peel.

96.2.6 Blanching

Blanching is applied to destroy the enzymatic activity of the vegetables and some fruits that are to be canned, dehydrated, or frozen. This operation is also combined with the peeling and/or washing in order to save, not only in investment and space costs, but also costs in energy. In the case of tomatoes, steam peeling simultaneously inactivates the pectinmethylesterase (PME) and pectinpolygalacturonase (PPG) enzymes (Cheftel and Cheftel 1992).

96.2.7 Manual Sorting

The peeled tomatoes are inspected before being placed into cans by trained workers who separate the tomatoes that do not meet the quality requirements which has been established by the Mexican standard for canned tomatoes. The tomatoes cannot be spotted, or rotten. The overly soft products are sent to the juice line.

96.2.8 Filling, Additives, and Containers

Filling the can is done mechanically or manually. The drained weight must be a minimum of 50% and the cans must be 90% filled (including the medium used as covering), leaving 10% headspace for expansion during thermal processing (CODEX STAN 13-1981). Because of the acid nature of the tomato, enameled cans and lids are used. The packing medium is juice or salted tomato puree. The pH must not be higher than 4.5. Organic acids may be added for adjustment. The most commonly used is citric acid. Sugar may also be added to compensate for the acid due to the added acid. Calcium chloride tablets are added to maintain the firmness of the tomatoes. The limit permitted in the end product is 0.045% (CODEX STAN 13-1981). The calcium ions react with the methoxy groups in the pectin chains, forming calcium pectate (Cheftel and Cheftel 1992).

96.2.9 Exhausting and Sealing

The vacuum within the cans and sealing is done at the same time. Generally, rotating automatic fillers controlled by an operator are used at a preprogrammed velocity, for example,

1000 cans/min (Fellows 1997). Juice or tomato puree at a minimum temperature of 86°C is added to the cans filled with hot peeled tomatoes that are then sealed. When the can is sealed, steam is injected into the headspace which creates a vacuum after it is cooled. This vacuum assures that the ends of the cans are flat or slightly concave during storage and will maintain the cans' integrity when made available to the consumer (Hersom 1991).

96.2.10 Thermal Processing (Canning)

The thermal canning process is undertaken in a rotating retort in a generally semicontinuous process at 100°C for 30–40 min because tomatoes are a high acid-food. The temperature and length of processing time depend on the product being canned, the size of the can and the type of retort. There are other thermal systems that involve a long tunnel through which a belt transports the products through the heating and cooling areas. The steam tunnels have the advantage of taking up less space and heating faster therefore requiring a shorter processing time. Once the cans leave the thermal process, they are cooled at 30–40°C to minimize quality loss. The steam in the headspace condenses at this stage and a vacuum is formed.

96.2.11 Quality Standards

The Processed Fruit and Vegetable Committee of the Codex Alimentarius FAO/WHO has a quality standard for canned tomatoes (CODEX STAN 13-1981) which includes six presentations: whole tomatoes, whole and sliced tomatoes, sliced tomatoes, diced tomatoes, and wedges. Table 96.4 indicates some of the specifications that must be met by these tomato products.

This international standard also includes specifications for quality, hygiene requirements, labeling, and absence of contaminants.

TABLE 96.4 Specifications for Canned Tomatoes.

Specification	Limit
Filling	Not less than 90%
Drained weight	Not less than 50%
Packing media	Tomato juice, tomato puree or tomato paste
Others ingredients:	
Onion, peppers, celery, basil leaf	Not exceeding in total 10% of the product
Salt	—
Sweeteners:	
Sucrose, dextrose, glucose syrup	When acidifying agents are used
Acidifying agents:	
Acetic acid, citric acid, lactic acid, malic acid, L-tartaric acid	Limited by Good Manufacturing Practice
Firming agents:	
Calcium chloride, calcium sulfate, calcium citrate, mono-calcium phosphate, calcium lactate, calcium gluconate	0.08% total calcium ion content in the end product in the styles "diced," "sliced," and "wedges," 0.045% total calcium ion content in the end product in the styles "wholes," "wholes and pieces," and "pieces."

Source: CODEX STAN 13-1981.

96.3 DICED TOMATOES: PROCESSING OPERATIONS

The operations for sorting and grading, washing, sorting, coring, and peeling for diced tomatoes are similar to the operations for whole canned tomatoes.

96.3.1 Size Reduction

Size reduction is a unit operation in which the average size of solid foods is reduced by the application of grinding, compression, or impact forces. Generally, one of these forces tends to be more important than the other two for the process in question. Peeled tomatoes are diced at an average size of $\frac{1}{2}$ or 1 inch. An inspection to eliminate blemished dices is made at this stage (Fig. 96.4).

96.3.2 Filling, Additives, and Containers

Enameled cans are mechanically filled with diced tomatoes. A maximum of 0.08% calcium chloride tablets are added during the filling operation to increase the firmness of the diced tomatoes (CODEX STAN 13-1981). Calcium can also be added by conveying the dices through a calcium bath. The packing medium is tomato puree with salt. The pH must not exceed 4.5.

The exhausting and sealing of the can and thermal processing is done in the same way as with whole canned tomatoes.

96.3.3 Quality Standards

Quality standard for canned tomatoes includes diced ones CODEX STAN 13-1981 (Table 96.4).

Figure 96.4 *Diced tomatoes.*

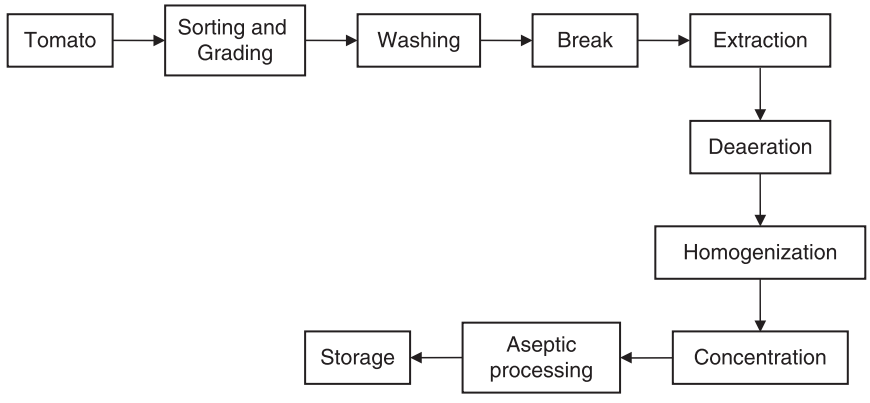

Figure 96.5 *Flow chart for tomato paste.*

96.4 TOMATO CONCENTRATE OR TOMATO PASTE: PROCESSING OPERATIONS

Tomato concentrate, or paste, is an important tomato product since it has great international demand. The paste is refined tomato pulp (juice) that is concentrated at 30–32°Bx or more (Fig. 96.5). This paste is used in sauces which can be marketed as such or as part of frozen meats and soups.

96.4.1 Sorting and Grading

The selection and classification is made by trained workers who take into consideration color, defects (worm damage, mechanical damage, anthracnose, fungi, and diseases) and soluble solids. The color is evaluated by the tomato's red surface or with a sample of tomato juice using a colorimeter. The industries generally apply specifications of quality to the raw material. Depending on the inspection results, the producer's lot is accepted or refused.

96.4.2 Washing

The tomatoes are washed in agitator tanks for 3–5 min to eliminate dust, fungi, insects, and drosophila eggs, and to reduce the microbial count. Some processing plants add chlorine to keep the concentration of spores low in the flume water. After completely eliminating the adhered dirt, the tomatoes are rinsed under water streams.

96.4.3 Break

Tomatoes for juice are processed by the hot break or cold break method. In the hot break method, the tomatoes are first chopped and then sent to the breaking up tank, which has a serpentine rotary to achieve uniform heating, where they are heated at 100°C. The hot pulp is pumped to the heating sterilizer where the pulp is heated up to 112°C to assure the inactivation of the pectinmethylesterase (PME) and polygalacturonase (PG) enzymes. The enzymes must be inactivated so that the juice will maintain its maximum viscosity. The juice is cooled within the tank to 100°C and then undergoes the extraction operation.

The largest juice production is obtained with the hot break method and is used to concentrate the paste with the high level of viscosity needed to prepare other tomato products such as sauces and catsup. Some breaking systems are done in a vacuum to reduce the degrading of the ascorbic acid.

The tomatoes are chopped and heated to 50–60°C in the cold break method to accelerate enzymatic activity. The juice thereby obtained has better color and taste, though less viscosity because of the activity of the enzymes. This low viscosity juice is adequate for the production of tomato juice, tomato soups, and dehydrated tomatoes.

96.4.4 Extraction

After the break system, the mashed tomatoes are sent to the extractor, pulper, or finisher to remove the seeds and skin. The extractor is a cylindrical perforated metal screen that has brushes or paddles inside which rotate at a high velocity. The tomato pulp is forced through the perforations in the screen by the brush action. The hole size of the screen determines the size of the pulp particles that affects the viscosity and texture.

96.4.5 Deaeration

Deaeration removes dissolved air incorporated in the flesh during breaking or extraction. This operation is done in a vacuum chamber. Foam is also prevented from forming during concentration. If the air is not eliminated, vitamin C is lost.

96.4.6 Homogenization

The juice is homogenized to increase viscosity and to reduce separation of the serum. The juice is forced through a narrow orifice at high pressure, shredding the suspended solids.

96.4.7 Paste Concentration

Concentration is the most important stage of the entire process. The water is partially removed from the juice through evaporation. The juice is pumped to the triple-effect evaporator where the puree is concentrated from 4.5% to 32% of the soluble solids (Bartholomai 1991). The triple effect vacuum system uses interconnected evaporators (effects); the steam obtained in an effect is directly used as a means of heating for the following effect. This steam can only be used so that the juice will boil at a lower temperature than the previous effect. In order for the difference between the juice temperature and the heating element to be maintained, the effects must function at progressively lower pressures.

The yield of the evaporators depends on a number of effects and brand. This can generally be between 300–600 ton/day of fresh tomatoes (Rossi Catelly T-30 and Manzini MV-1500, respectively). The juice also is concentrated to paste by inverse osmosis with yield of 350 ton/day of fresh tomatoes.

96.4.8 Aseptic Processing

The paste is pumped to a deposit tank and from there to a scraped surface or tube-in-tube heat exchanger where pasteurization takes place at 110°C for 2 min or at 96°C for 3 min (Barringer 2004). Afterwards the product is cooled (30°C) and filled into sterile containers

TABLE 96.5 Physical and Chemical Specifications for Tomato Paste.

Specifications	Limit	
Color	Minimum in Munsell units	
	Number disc	Area exposed
	1. Red	53%
	2. Yellow	28%
	3. Black	19%
	4. Grey	19%
	3. and 4.	9.5% and 9.5%
Soluble solids free of salt	Minimum	25%
Acidity (citric acid)	Maximum	1.0%
Chloride (NaCl)	Maximum	2.0%
pH	4.0–4.5	

Source: NMX-F-025-1982.

in aseptic filler. Pasteurization occurs before it is packed; after the thermal treatment, the product is cooled to conserve its color, aroma, and taste qualities. The aseptic containers are not resistant to heat higher than 38°C. The container used industrially is an aseptic bag of 235 kg within a metallic drum or an aseptic bag of 1250 kg located within a wooden bin. The shelf life of these products is two years at room temperature.

The Manzini aseptic fillers work at the rate of 10–12 ton/h of tomato concentrate, while the Flash Cooler aseptic fillers do so at 23 ton/h of tomato concentrate.

96.4.9 Quality Standards

96.4.9.1 Mexican Quality Standard. The Mexican standard of quality for packed tomato paste (NMX-F-025-1982) takes into consideration sensory, physical and chemical specifications (Table 96.5), microbiological and chemical contaminants, methods of analysis and sampling, labeling, container and packing.

TABLE 96.6 Specifications for Tomato Concentrates.

Specifications	Limit
Product designation:	
Tomato puree	Tomato concentrate that contains not less that 8%, but less than 24%, of natural tomato soluble solids.
Tomato paste	Tomato concentrate that contains 24% or more of natural tomato soluble solids.
Permitted ingredients:	
Salt, spices, natural vegetable products (onion, basil leaf, etc.)	—
Acidulants:	
Lemon juice	—
Sugars or other sweeteners	Not permitted
pH regulating agents:	
Sodium hydrogen carbonate	To raise the pH to a level not above 4.3
Citric acid, malic acid, lactic acid, L-tartaric acid	To maintain the pH at a level not below 4.3

Source: CODEX STAN 57-1981.

There also exists a Mexican standard for packed tomato puree (NMX-F-033-1982), which takes into account tomato puree containing a minimum of 10% of salt free soluble solids.

96.4.9.2 International Quality Standard. The Codex standard for tomato concentrates CODEX STAN 57-1981 (Table 96.6) covers only tomato puree and tomato paste. That is to say, it does not consider tomato sauce, chili sauces or catsup since they are seasoned in different concentrations and contain black pepper, onion, vinegar, sugar, and so on, in quantities that change the taste, aroma, and flavor of the tomato ingredients.

The tomato concentrate must have 8.0% or more of natural soluble solids without reaching the state of dehydration. The Codex standard determines the product type according to the percentage of natural soluble solids.

This international standard also includes the sampling criteria used for natural soluble solids, quality specifications, hygiene requirements, labeling, and limits for contaminants. The processors for tomato products handle the specifications shown in Table 96.7.

96.5 TOMATO JUICE: PROCESSING OPERATIONS

Juice is an intermediate product in the processing of tomato paste since it is obtained in the juice extraction stage that will, when the process continues, later result in tomato concentrate.

The tomatoes for juice making are selected, classified, and washed just as those used for tomato paste are.

96.5.1 Break

The tomatoes are chopped and heated to 50–60°C (cold break; Gould 1992). The juice obtained with this method has a better color and taste, though less viscosity due to the enzymatic activity. This juice with less viscosity is adequate for preparing tomato juice. The chopped tomatoes are heated at 83°C for 15 s to deactivate the enzymes in order to obtain juice with more viscosity (Figuerola 1992).

96.5.2 Extraction

After the breaking up system, the ground tomatoes are put through an extraction to remove the seeds and skin in a screen with paddles or pulper.

TABLE 96.7 Commercial Specifications for Tomato Paste.

Presentation	Soluble Solids °Bx	Texture (Screen)	Consistency (12°Bx, 20°C, 30 s) cm	Color (Hunter Lab)	pH
Hot break	30–32	0.033″/0.039″/0.045″	4.5–6.0	Fancy USA	4.1–4.5
Cold break	36–38	0.024″/0.033″	8.0–11.0	>2.2	4.1–4.5

96.5.3 Deaeration

Air is removed from the juice in a vacuum chamber to eliminate the oxygen dissolved in the product.

96.5.4 Formulation

The juice is formulated at this stage according to the characteristics demanded by the market: the most common is juice with salt and another more complex presentation with different spices. Organic acids, the most common being citric acid, is added to adjust the pH.

96.5.5 Homogenization

Homogenization retards the separation of the solids and liquid. The juice is forced through a narrow orifice at a pressure of 1000–1500 psi at a temperature of 66°C to shred the suspended solids (Gould 1992).

96.5.6 Aseptic Processing

The tomato juice is pasteurized at 130°C for 4–5 s. This short thermal treatment at a high temperature (high temperature-short time) is very efficient since it preserves the nutritional and sensory properties of the product. The heating and cooling takes place in the scraped surface heat exchanger or tubes. The cooling temperature is between 85–90°C.

The juice must be cooled to a temperature lower than 30°C for aseptic filling to take place. A 500 or 1000 mL capacity tetrabrik is used for packing. The container has six layers with an external layer and an internal (which is in contact with the product): (1) the external layer made of polyethylene, (2) paper and decoration, (3) polyethylene, (4) aluminum, (5) and (6) polyethylene (Richardson and Selman 1994). The combination of materials is especially adapted to preserve the sensory characteristics of the product and to prevent light and air from entering. The paper layer (2) makes the package rigid, while the aluminum layer (4) acts as an efficient barrier against oxygen and light. The polyethylene prevents leakage and permits the package to close well. The name of the company, information about the product, drawings, recommendations, and so on, is printed on the paper layer instead of a big label which is then protected by a polyethylene layer (1). At the aseptic filler, the material is shaped into a closed longitudinal roll under sterile conditions. The juice is added and the container is closed below the level of the liquid so that the package is now completely filled; the jaws cut and separate them. The packing is sterilized with peroxide (H_2O_2).

96.6 TOMATO CATSUP: PROCESSING OPERATIONS

This sauce is a product that is made with different vegetables, spices, and vinegar. It is used as a complimentary seasoning in everyday food. There are sauces in every country that vary according to its habits. Nevertheless, catsup is very well known. This product can be made from fresh tomatoes or from tomato paste, the latter being what is used industrially. The process consists of the mixing of tomato paste (30–32°Bx) with salt,

TABLE 96.8 Physical and Chemical Specifications for Tomato Catsup Sauce.

Specifications	Limit
Color, minimum (Munsell units)	Acceptable
Consistency, maximum (cm)	12
Moisture, maximum (%)	73
Total solids, minimum (%)	27
Protein, minimum (%)	1.5
Acidity as acetic acid, maximum (%)	2.5
Fiber, minimum (%)	0.4
Ash, maximum (%)	4.5
Total reducing, maximum (%)	24
Chloride as NaCl, maximum (%)	4
Pectin (dry base), maximum (%)	3.5
pH, maximum	4.3

Source: NMX-F-346-1980.

sugar, onions, vinegar, black pepper, mustard, mushroom, and bell peppers. The mixture is pasteurized at 96°C for 4–6 min. The pasteurization temperature varies depending on the equipment used and the end product desired. The sauce then goes through the stage of deaeration, after which it is cooled to filling temperature (25–75°C). The filling is done while hot in order to pasteurize the contents of the sealed container. The Mexican standard NMX-F-346-1980 for tomato catsup establishes the physical and chemical specifications shown in Table 96.8.

This Mexican standard also includes microbiological specifications (maximum limits of mesophyll aerobic bacteria and fungi), for permitted food additives, acidifying agents, sampling criteria, quality specifications, hygiene requirements, labeling, and storage.

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97

Dried Tomato

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97.1 Introduction 1109
97.2 Nutritional Aspects 1111
97.3 Processing 1113
97.3.1 Pretreatments 1113
97.3.1.1 Washing 1114
97.3.1.2 Cutting and Seeds Removing 1114
97.3.1.3 Osmotic Dehydration 1114
97.3.1.4 Application of Antioxidants 1114
97.3.1.5 Effect of Pretreatments 1115
97.3.2 Sun and Solar Drying 1115
97.3.3 Convective Air Drying 1116
97.3.4 Spray Drying 1117
97.3.4.1 Principles 1117
97.3.4.2 Spray Drying of Tomato 1118
97.4 Packaging 1119
References 1120

97.1 INTRODUCTION

Drying is one of the oldest methods of preserving food products. Dried foods have a good preservation capacity because the moisture content is so low that spoilage organisms cannot grow and enzymes become inactive. Apart from its preserving function, drying also has other advantages, such as providing a wide variety of food products and avoiding the need of much storage space and refrigerating systems (Hayashi 1989).

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Vegetables are a good source of minerals and vitamins, in particular thiamine (B1), riboflavin (B2), and niacine (B_3), and also provide useful amounts of the fiber (bulk) needed for a good functioning of the intestine (Multon 1992).

Vegetables that are traditionally dried include peas, corn, peppers, zucchini, okra, onions, tomatoes, and green beans. Usually vegetables are cut into pieces of suitable size before drying, since the increase in the superficial area will fasten significantly the drying process.

Almost all vegetables need to be blanched before drying, by scalding in boiling water for a short period of time. This operation stops the enzyme action, which drying cannot stop. In fact, if vegetables are not blanched, enzymes will destroy the color and flavor during drying and storage. However, when blanching is done in boiling water, some water soluble nutrients are lost in the process. To minimize these losses, steam blanching can be used although it is a slower process. The time of blanching must be carefully selected in order to minimize the losses in nutrients and ensure that the enzymes are effectively inactivated. In the case of tomatoes, blanching is not essential because of the richness of tomatoes in antioxidant substances.

The tomato (*Lycopersicon esculentum Mill.*) is native from South America, and is presently one of the most important crops grown worldwide. Mostly, tomatoes are consumed either fresh as salad or in food preparations. However, and since it is highly perishable, they are also used in the form of juice and canned varieties and in the manufacture of various products, such as puree, paste, ketchup, sauce, and soups, among others (Sogi and others 2003; Akanbi and Adeyemi 2006).

The dehydration of tomato has been practiced for many years as a means of preserving it, and offering an alternative way of commercialization. The most popular method used to dry tomatoes is convective hot air drying. However, this method has some important disadvantages related to physic and structural changes, such as migration of soluble solids, shrinkage, case hardening, loss of volatiles, and aroma. Other drying methods are used, that minimize the effects described earlier: freeze drying, air drying at low temperature, and vacuum drying (Akanbi and Adeyemi 2006). Some experiments were carried out to process tomatoes by the foam-mat technique (Ginette and others 1963) or by spray drying (Olorunda and others 1990; Zanoni and others 1999).

The drying, as well as the predrying treatments can affect substantially the quality of the final product. Also storage-induced changes affect the quality of dried products. Some of these involve alterations in the physical state of the material, like shrinking of cells, loss of rehydration ability, wettability, migration of solids, case hardening, and loss of volatile aroma components. On the other hand, chemical modifications, such as enzymatic reactions, nonenzymatic browning and oxidation of lipids, pigments, and vitamins are also affected by physical structure, mainly due to its influence on molecular mobility, which changes diffusivity of the relevant molecular species (Karel 1991; Nijhuis and others 1998).

The increasing consumer demand for processed products that preserve in a better way their original characteristics, as well as the higher industrial use of dried products, has increased the level of requirements, particularly in terms of "functional properties." Telis and Sobral (2002) studied the glass transitions of freeze-dried and air-dried tomatoes and concluded that for the osmotically treated, air-dried tomatoes, the glass transition temperatures were lower than those of the freeze-dried product. Moreover, no differences were observed between glass transition temperatures of samples dried at different air temperatures.

97.2 NUTRITIONAL ASPECTS

Tomatoes are low caloric (19 kcal/100 g of edible part) (Senser and others 1999) and rich in vitamins and minerals, making it a good food product from the nutritional point of view (see Tables 97.1 and 97.2).

Tomato contains not only the nutritional antioxidants like vitamins C and E, but also important quantities of nonnutritional antioxidants like β-carotene, carotenoid, flavenoids, flavone, and total phenolics compounds, and so on.

Some epidemiological studies reveal that the consumption of tomato and tomato-based products can help to prevent various forms of cancers (especially prostate, lung, pancreas, stomach, or digestive-tract cancers) as well as heart diseases (Rao and Agarwal 2000; Chang and others 2005; Toor and Savage 2006). This is the main reason for the increasing interest in the antioxidant components of tomatoes, such as lycopene, ascorbic acid, phenolics, and flavonoids, and the demand for ready-to-use products, which have similar health benefits to the original raw products (Dewanto and others 2002; Schieber and Carle 2005; Toor and Savage 2006).

Fresh tomatoes can be dried as halves, slices, quarters and powders, that are lately used as components for pizza, pestos, snacks, and various vegetable dishes (Lewicki and others 2002). Industrial processing of tomatoes to a final moisture content of less than 15% often involves high temperatures (ranging from 60°C to 110°C) for a period of 2–10 h in the presence of oxygen, and therefore, the products show some oxidative damage (Zanoni and others 1999).

Lavelli and others (1999) reported a reduction in the vitamin C content of tomatoes in drying and Dewanto and others (2002) have observed important losses of ascorbic acid during the production of dried tomato halves and tomato pulp using high temperatures. However, the losses of ascorbic acid are not only dependent on the drying temperatures used but also on the moisture content in the final product.

Air drying of tomatoes also leads to an increase in the 5-hydroxymethyl-2-furfural (HMF) content, which results in undesirable color and appearance changes of dried tomatoes (Zanoni and others 1999). It has been reported by Olorunda and others (1990) that for the air drying of tomatoes an increase in drying time and temperature result in tissue darkening, while other studies report an increase in darkness and decrease in redness (Kerkhofs 2003).

Lycopene is a C_{40} carotenoid polyene, and is the principal coloring matter responsible for the deep-red color that characterizes the ripe tomatoes and tomato products. However, its importance goes beyond its coloring properties, since its presence is associated with recognized health benefits. Although it has no provitamin A activity, lycopene is able to function as an antioxidant and to quench singlet oxygen in vitro. The quenching constant of lycopene

TABLE 97.1 Nutritional Composition of Tomatoes

Component	Composition (g/100 g of Edible Part)[a]
Water	94.2
Protein	1.0
Fat	0.2
Carbohydrates	3.0
Organic acids	0.5
Fiber	1.8
Minerals	0.6

[a]In tomatoes the edible part is 85% of the fruit (Ferreira 1977).

Source: Senser and others (1999).

TABLE 97.2 Amounts of Vitamins and Minerals Present in Tomatoes

Component	Composition (Per 100 g of Edible Part)
Vitamins	
Carotenes	820 μg
Vitamin B_1 (thiamine)	55 μg
Vitamin B_2 (riboflavin)	35 μg
Vitamin B_5 (pantothenic acid)	310 μg
Vitamin B_6 (pyridoxine, pyridoxal phosphate)	100 μg
Vitamin B_9 (folic acid)	40 μg
Vitamin C (ascorbic acid)	25 mg
Vitamin E (alpha-tocopherol)	800 μg
Vitamin H (biotin)	4 μg
Vitamin K (phytonadione)	8 μg
Vitamin PP (nicotinamide)	530 μg
Minerals	
Calcium	14 mg
Chlorine	60 mg
Copper	90 μg
Fluorine	25 μg
Iodine	2 μg
Iron	500 μg
Magnesium	20 mg
Manganese	140 μg
Phosphorus	25 mg
Potassium	295 mg
Selenium	1–10 μg
Sodium	6 mg
Zinc	240 μg

Source: Senser and others (1999).

is more than two times that of β-carotene and more than 10 times that of α-tocoferol (Shi and others 1999; Goula and Adamopoulos 2005). The heating, and in particular the drying of tomato products under different processing conditions to manufacture tomato juice, pulp, powder or others, may cause a more or less important degradation of lycopene, according to the conditions used. The principal causes for lycopene degradation are isomerization and oxidation (Goula and Adamopoulos 2006). However, air drying is reported to have little effect on the lycopene content of tomatoes (Kerkhofs 2003).

The total phenolics and carotenoids of tomato have seem to be quite stable during processing under high temperature conditions, and thermal processing has been reported to release more bound phenolics due to the breakdown of cellular constituents (Dewanto and others 2002).

Oxidation increases during dehydration if the moisture content falls below the monolayer moisture content of the product (Zanoni and others 2000). To reduce oxidative damage in the final dried product, Zanoni and others (1999) propose a modification in the operating conditions during air drying of tomatoes, by using low temperature treatments, reducing tomato thickness (using tomato slices or quarters), and partial removal of water (producing tomatoes with an intermediate moisture content).

Color is better retained with low temperature treatments (Kerkhofs 2003), and for osmotic dehydration (Shi and others 1999). However, it is not known whether color and

the antioxidant components of tomatoes would be retained in a higher degree if lower temperatures were used and a higher moisture content product was produced.

Chang and others (2005) reported that the drying process could enhance the nutritional value of tomatoes by increasing parts of the total flavonoids, total phenolics, and lycopene contents. This is a demystification of the idea that the nutritional value of processed foods is lower than that of the fresh ones. Therefore, a proper processing to develop a new type of product could somehow be considered helpful to promote the nutritional value and extend the utilization of tomato. In fact, tomato could be regarded as source of food additives used for fortification and stabilization, even when submitted to technological processes that can eventually cause some degree of loss in the more labile hydrophilic antioxidants. From the economic point of view, the extract products from dried tomato could be further developed as food additives useful in other food applications such as instant food products.

Recently, a new method to produce semidried tomatoes has been developed by drying at 42°C for 18 h to a final dry matter content of about 19%. The bright-red semidried tomatoes obtained are then packed in airtight plastic bags after the addition of canola oil to extend their shelf-life. It is believed that these semidried tomatoes would retain high levels of antioxidant compounds.

97.3 PROCESSING

Tomatoes, like other vegetables, can be dried using various methods. However, in any technique used to dry tomato the time required for drying depends on many different parameters, some related to the product and others related to the drying conditions. As to the first, it is worth mentioning the tomato variety and its composition, in particular the soluble solids content (°Brix) of the fresh product. As to the second, the most important factors are: the size of the tomato segments, the air humidity, the air temperature, and velocity, and the efficiency of the drying system. As expected, the rate of drying directly influences the final quality of the dried product.

In general, dried tomatoes undergo the following process steps: predrying treatments (such as size selection, washing and tray placing), drying or dehydration, and postdehydration treatments (such as inspection, screening, and packaging).

The traditional sun-drying method is advantageous due to its simplicity and low cost, but on the other hand, it requires long drying times and has some disadvantages that can compromise the product quality, like the possibility of contamination from dust and insects or suffering from enzyme and microbial activity. As to industrial drying, when high temperatures are used (~90°C) the tomatoes loose quality regarding color and aroma and may suffer from case hardening (the formation of a hard outer shell), offering a resistance to moisture diffusion and impeding the drying of the interior part of the product. Therefore, the ideal conditions for drying tomatoes are mild temperatures (between 45°C and 55°C), because they allow the dried product to retain its nutrients (including vitamins and lycopene) and flavors.

97.3.1 Pretreatments

Before drying, tomatoes are peeled, mostly by dipping in boiling water, cut and then subjected to different pretreatments.

97.3.1.1 Washing. For small and medium production scales, and when the tomatoes are not peeled, washing can be done in washers by immersion, on three stages. For tomatoes, in the first washing the ideal is to immerse them for 20 min in a solution with 80 mg/L of chlorine. After this first bath, the tomatoes are placed in a second tank where the remaining impurities are removed. This second wash should last about 10 min and the water should be treated with a concentration of 50 mg/L of chlorine. The third and final washing stage is carried out with pure water, without the addiction of chlorine.

97.3.1.2 Cutting and Seeds Removing. Depending on the final usage of tomatoes and the drying method used, they may be cut in halves in the longitudinal direction, using stainless steel knives. Alternatively they may be cut in smaller portions.

The seeds are removed at this stage and those tomatoes not obeying quality standards must be rejected.

97.3.1.3 Osmotic Dehydration. The osmotic dehydration is a pretreatment used to minimize the decrease of nutrients (such as lycopene and vitamin C), allowing the retention of these compounds in the latter convective drying. This procedure has some important advantages, such as low energy requirements, the obtaining of a final product with better appearance (in particular in respect to color), the possibility of incorporating some components that may increase the preservation capacity and minimize the changes caused to color, texture, and flavor by the heat. These reasons contribute for the increase of interest for osmotic dehydration as a pretreatment for the processing of tomato in the most recent years.

This operation consists in the immersion of the product in a solution with a water activity lower than that of the food, which causes the dehydration of the product and at the same time some alteration in its composition. For the osmotic dehydration of tomato, the residual salt amount must be defined according to the products already commercialized, or in agreement with the demands of a specific client. Usually, the tomatoes are left for 30 min in a solution with 5% (w/w) of salt. Alternatively, the pretreatment can be done using sugar mixed with the salt, thus obtaining tomatoes with better organoleptic characteristics. For this, a 65°Brix syrup with one part of salt for 10 parts of sugar is used, at 45°C.

After this pretreatment, the tomatoes are quickly washed with potable water and are drained for 2 min for removal of the syrup in excess.

97.3.1.4 Application of Antioxidants. This treatment intends to minimize the oxidative processes during drying, and is done by immersing in a bath with ascorbic acid (1500 mg/L) and metabisulphite (100 mg/L) for 1 min. Although there is a general tendency to minimize or even to avoid the usage of metabisulphite, it is known that when it is used in small quantities together with the ascorbic acid, its effect is maximized, being more efficient than the two used separately, even in higher concentrations.

Other pretreatments include dipping in calcium chloride solution (Collins and others 1997), sodium chloride solution (Olorunda and others 1990; Sacilik and others 2006), sulphited starch solution (Tripathi and Nath 1989), or osmotic dewatering (Shi and others 1999). In other cases, tomatoes were just cut into halves, quarters or slices and subject to drying (Hawlader and others 1991; Zanoni and others 1999).

97.3.1.5 Effect of Pretreatments. The color of tomatoes dried in pieces is little affected by storage (Tripathi and Nath 1989). However, the powdered product is unstable. It was found that calcium chloride greatly increases the losses in carotenoid, but on the other hand, minimizes browning. Contrarily, the use of sodium chloride and metabisulphite has no effect on the browning of the powder (Baloch and others 1997). As to the effect on the carotenoid losses, sodium chloride has no effect, while metabisulphite decreases the losses.

Osmotic pretreatment proceeding air drying is found to be advantageous for quality of the product (Collins and others 1997; Shi and others 1999). Lycopene loss and isomerization are reduced compared to the conventional air drying process, and the final product properties are similar to those of typical dried fruits.

97.3.2 Sun and Solar Drying

Traditionally, farmers and housewives dried meaty tomatoes with few seeds. Tomatoes with these characteristics dry more quickly than other varieties while having great flavour. The drying of the excess production adds value and shelf-life to tomatoes while taking advantage of the off-season markets. Ripened tomatoes are firstly washed and halved and then they are spread into drying trays in the sun for 5–10 days. The result is a product with typically 12–24% moisture, robust in taste, and which darkens after some time (9–12 months). Products dried by this method require continuous supervision and do not allow homogeneity in the quality of the final product. However, the solar energy can be used without stringent conditions for drying purposes and overcomes many of the sun drying problems.

It is commonly known that the drying process can be conducted by using several solar drying methods. However, solar dryers with solar heater air provide a better control of the required drying air conditions. In addition, solar tunnel dryers, based on plastic tunnel greenhouses, have a good efficiency and do not require any other energy during the operation. Sacilik and others (2006) describe a solar tunnel which is covered by a plastic film of semitransparent polyethylene with 150 μm thickness, in order to incorporate UV and far infrared protection. Wire mesh trays are used to accommodate the tomato halves during the drying process. To compare the performance of the solar tunnel dryer with that of open sun drying the authors used control samples of tomatoes put on a tray near the dryer and dried simultaneously under the same weather conditions. The time to reach a final moisture content of 11.50% (w/w) in the solar tunnel was found to be between 82 and 96 h, while the drying time for open sun drying varied from 106 to 120 h. The higher drying rate obtained when using the solar tunnel, and compared with the open sun drying, could be explained by the temperature difference (more 12.6°C) and relative humidity differential (less 8.7%). In addition, the dried solar tunnel tomato samples had redder and lighter colour than those of the open sun drying.

Other design of a solar drying, a staircase solar dryer, was characterized by Hallak and others (1996) to dry tomato. Basically, it has a shape of metal staircase with its base and sides covered with double-walled galvanized metal sheets and the upper surface covered with a transparent polycarbon sheet. The air motion by natural convection is assured by air-entry points located in the base of the dryer and an exit through a chimney. The temperature inside the dryer must not exceed 70°C, to prevent cooking, and the time necessary to reach a moisture content of 19% is between 2.5 and 3.5 days, very fast when compared to the sun drying.

Sharma and others (1995) present the cabinet-type and multistacked natural convection solar dryers and, additionally, they also analyse a multishelf forced convection solar dryer for drying tomatoes, in which the air temperatures are between 40°C and 65°C along a drying period of 9–16 h. Although an indirect-type solar dryer needs a higher upfront investment cost and availability of electric power, this type of system presents a better performance when compared to the others, showing a higher efficiency and allowing the drying of a higher quantity of material over a given drying period. Moreover, the system can be improved on a semi-industrial scale and operated in a continuous mode trough an auxiliary energy resource.

Although it is true that the direct open-air sun exposure drying processes are the cheapest methods, their application for rural and commercial scales is limited to climates with hot sun and dry atmosphere, as well as with strong winds. Thus, these methods of preservation have been substituted by indirect drying methods using solar heated air.

97.3.3 Convective Air Drying

For the industrial drying, the tomatoes are distributed over trays with a load of approximately 8 kg/m^2. The control tray must have the same load, since it will be used to accompany the process and identify the end-point of drying.

The temperature of the drying air must be set to 60–65°C and the trays must be turned 180° every hour in order to reduce the drying time and to obtain a uniform final water content.

The end of the drying is when the weight of the product obeys the relation:

$$W_f = (W_s - W_i * X_i)/X_f$$

where W_f = final weight in the dryer; W_i = initial weight in the dryer; X_i = initial moisture content; X_f = final moisture content (desired).

Andritsos and others (2003) describe a system for the dehydration of tomato in a tunnel with convective air drying. The tomato dehydration process can be divided into three stages: a predrying preparation step, the drying step, and the postdrying treatment. In the predrying treatment the raw tomatoes are prepared for the dehydration process. This step involves firstly the selection of the tomatoes, in respect to their maturity and soundness (about 40–70% of the tomatoes are selected to proceed for drying depending mainly upon the climatic condition during tomato growth and harvesting). Then, the tomatoes are sorted into two sizes: tomatoes above 90 g and tomatoes of lower weight. Posterior, the tomatoes are placed in crates, washed to remove dust, dirt, plant parts, and so on, and finally cut into two halves and placed into stainless steel trays (mesh type, 100 × 50 cm^2).

The drying operation is carried out in a tunnel drier. This drying system consists of the following main components (Andritsos and others 2003):

- *Finned-tube coil air-water heat exchanger* (INTERKLIMA) for heating the drying air and having a capacity of 300,000 kcal.
- *Fan units.* Two fan units installed in the system, totaling a rated power of 7 kW. The drying system, operated with an air flow rate in the tunnel between 10,000 and 12,000 m^3/h, corresponding to a superficial air velocity of 1.7 m/s (without the trays loaded with product), used approximately 30% of its power capacity. However, in the presence of the loaded trays, which block partially the cross-section

of the tunnel the air velocity increases by 20–50%, depending on the location inside the tunnel.

- *Drying tunnel*. The rectangular tunnel (width a cross section of 1 × 2 m) is 14 m long and is constructed of polyurethane aluminum panels. In the tunnel, the heated air flows in counter current in respect to the movement of the trays. The trays loaded with tomato are placed at the entry of the tunnel and they are conveyed towards the end (where the hot air enters the tunnel) in a semicontinuous way: approximately every 45 min a series of 25 trays with dried product is removed from the end of the tunnel and 25 trays loaded with raw tomatoes are inserted at the entry, pushing the upstream trays towards the end. Each tray is loaded with approximately 7 kg of raw tomatoes. The temperature profile along the height of the tunnel is uniform, allowing the obtaining of a uniform product regardless of the tray position.
- *Measuring instruments.* The inlet and outlet temperatures are continuously monitored using thermocouples. The moisture content is measured by weighing certain marked trays at various locations in the tunnel.

The postdehydration step involves inspection and screening (the removal of dehydrated pieces of unwanted size, of foreign materials, etc.) and packaging in glass jars with olive or sunflower oil, wine vinegar, salt, garlic, and various herbs.

The weight of the processed product reduced about 10–12 times after drying, thus obtaining tomatoes with a moisture content of about 10% (w/w). The removal of the moisture content appears to be faster at the first half part of the tunnel. The residence time of the product in the drier is 30 h, and during that period about 4200 kg of raw tomatoes are introduced in the tunnel and the production of dried tomatoes reaches about 400 kg (Andritsos and others 2003).

Alternatively, the drying of tomato can be done with a wide variety of conditions. Hawlader and others (1991) dried tomato slices at temperatures between 40°C and 80°C with an air flow ranging from 0.7 to 1.8 m/s. Shi and others (1999) used higher temperatures (95°C), Olorunda and others (1990) used temperatures between 60°C and 80°C, Zanoni and others (1999) dried tomato halves at 80°C and 110°C and Lewicki and others (2002) dried tomato quarters at 60°C with an air velocity of 2 m/s. The drying times varied from a few hours to 10 h.

97.3.4 Spray Drying

Spray drying is an important method to dry heat sensitive products such as fruits and vegetables. It is a relatively efficient method that allows the conservation of the purity of the food particles. For the production of tomato powder by spray drying, is recommended the use of tomatoes with bright red color, thick walls, high solids, and pectin content.

97.3.4.1 Principles. The spray drying operation consists in the evaporation of moisture by atomization of a feed into a spray which contacts with a drying medium (usually hot air). The products that can be spray dried follow into two major categories: nonsticky and sticky products. The sticky products are normally more difficult to spray dry, because during the drying process they may remain as syrup or stick on the dryer wall, or even form unwanted agglomerates in the dryer chamber and conveying system resulting in lower product yields and operating problems. On the contrary, nonsticky products can

be dried using a simpler dryer design and the powder obtained is relatively less hygroscopic and more free flowing. Fruit and vegetable juice powders, honey powders, and amorphous lactose powder, are some examples of sticky products.

The problem of powder stickiness is mainly related to the low glass transition temperature (Tg) of the low molecular weight sugars present in such products, (mainly sucrose, glucose, and fructose) (Roos and others 1996). Tomato pulp is a typical example of a product that is very difficult to be spray dried due to the low glass transition temperature of the low molecular weight sugars present. The sugars found in tomato products are essentially dextrose and levulose with a Tg of 31 and 5°C, respectively (Bhandari and others 1997).

During spray drying, different amounts of additives are used depending upon the specific product in use. Tomato is most commonly used and can be dried with or without additives. Spray drying seems to be the most efficient process in obtaining high quality tomato powder at a low cost (Masters 1976).

Centrifugal or spinning disk type atomizers are the most adequate for fruit juices with discrete pieces of pulp (Van Arsdel and others 1973). Industrial dryers usually have multinozzles to deal with the high feed rate. It is important to consider the ease of accessing and removing the nozzles, uniformity of distribution, and their visibility (Barbosa-Canovas and Vega-Mercado 1996).

Special attention must be given to the chamber design, to the handling of the dried particles, and to the packaging of the product during drying, because of the thermoplastic and hygroscopic characteristics of fruits and vegetables.

97.3.4.2 Spray Drying of Tomato. According to Van Arsdel and others (1973), the best tomatoes for dehydration are those with thick walls, bright red color, and high solids and pectin content.

Fresh, ripe tomatoes are soaked in a vat and then transported by a rolling conveyor to a spray-washing vat. After this washing operation, the tomatoes are manually sorted and crushed in a chopper to obtain the pulp.

Tomato is spray-dried in large amounts from a concentrated paste. The powder is made from "hot break" paste or "cold break" paste. If the "hot break" paste is used, the tomatoes are firstly heated at 85–90°C and then crushed, and the seeds and skin are removed before refinement of particle size. However, Noyes (1969) refers that tomato puree which contains skin and seeds originates a more dryable product, recommending grinding the seeds to 325 μm. When a "cold break" paste is used, tomato pulp is held for a few seconds in order to obtain pectin decomposition, which will provide an easier-to-spread paste.

Usually, the powder obtained from the "hot break" is more desirable. The juice is then concentrated in a double effect evaporator and this product goes to a feeding tank to be pumped to the spray drying (Masters 1976). "Cold break" tomato pastes are spray dried at higher concentrations than "hot break" pastes (50°Brix for "cold break" and 45°Brix for "hot break").

A spray drying plant capable of producing a free-flowing product that when reconstituted compares favorably with tomato paste consists of a cocurrent drying chamber with a jacketed wall for air-cooling. If moderate drying air temperatures are used, the intake of cool air is controlled in order to maintain a desired temperature in the range 38–50°C.

The tomato heavy paste goes to a rotatory atomizer containing several vanes. The paste is sprayed into a stream of hot air (138–150°C) and then cooled (Gransmith 1971; Jayaraman and Das Gupta 1995). The cool chamber wall is favorable to minimize the

thermoplastic particles from sticking, because the outer surface of the thermoplastic particles coming in contact with the cold wall solidifies. This method, although improves the process, does not solve the problem, because the cold chamber wall also cools the surrounding environment and causes an increase in the relative humidity of the air close to the wall surface. Droplets of 600–800 μm are desirable for tomatoes with 28% solids constituents.

An alternative approach to the processes described earlier is the Birs Tower process, in which the drying takes place in a very tall tower into which the tomato juice is introduced as a spray at a predetermined height. The whole drying process relies on the time-delayed fall of the product droplets and the very low temperature (not exceeding 30°C) of the upward air flow. In this way the droplet expansion takes place very slowly and explosion-type evaporation is avoided. Therefore, the particles are not exposed to high temperatures likely to damage their organoleptic properties (Gransmith 1971). The volatile compounds present in the paste are better retained as well as the quality of tomato solids. However, the cost of building and operating such towers is very high (Bhandari and others 1997). The tomato powder produced has 10% moisture content, which is later reduced by using a fluidized bed attached to the base of the dryer (Masters 1976).

Another system capable of producing a free-flowing product is a scraped surface drying chamber. This method can be very useful for relatively less thermoplastic sugar such as lactose or sucrose. Karatas (1989) developed an experimental spray dryer with a chamber wall scraper specifically to dry tomato juice. The product recovery was up to 77% with a low inlet air temperature (115°C).

According to Karatas and Esin (1994), during air drying of tomato concentrate droplets the constant rate period lasts about half a minute. When the drying of the surface ends, the diffusional falling rate period shows discontinuities, with the drying rate falling to zero occasionally and then restarting with a drying rate different from the previous period. This behavior is a typical consequence of case hardening phenomena. When the rate of moisture migration from inside of the particle towards the surface becomes lower than the rate of the moisture evaporation from the surface, the surface becomes very dry and hard. Thus, a thin layer is formed, and since it is not permeable to water locks in the remaining moisture inside the particle or the drop. Therefore, drying stops and the average moisture content becomes constant. Though there is no net drying during the case hardened state, internal diffusion continues, and since the rate of output is zero, moisture accumulates in the hardened volume and plasticizing action proceeds outward. When the surface layer is plasticized to a sufficient degree, drying restarts and persists until the rate of internal supply plus moisture accumulated in the surface shell volume can meet the drying rate. To provide a smooth drying operation the drying rate must be low, which can be achieved either by operating at low temperatures or by increasing the moisture content of the drying air, or even a combination of both in order to keep the surface of the drying material plasticized (Karatas and Esin 1994; Szentmarjay and others 1996; Goula and Adamopoulos 2005a,b). However, prolonged drying could cause severe degradation in the sensory and the nutritional properties of the tomato.

97.4 PACKAGING

The tomato powder is packaged in an air-conditioned packing room (Masters 1976). Lumpiness decreases as cooling powder increases. However, the maximum powder temperature to

obtain a lump-free product during storage depends upon the type of the tomato. If the product is used within a few months and it is atmosphere packaged in dry air at low temperature, the product will suffice. Since tomato powder cannot contain more than 2% moisture content, nitrogen or carbon dioxide atmosphere packaging is the most appropriated. For this reason low moisture content of dried fruit juices is required for storage. In order to prevent lumpiness noncaking agents can be used: food gel, silica gel and other noncaking additives can be added to prevent caking (Van Arsdel and others 1973).

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Volume 2 Index

2-Acetyl-1-pyrroline, 294, 727–728, 732, 938–939
2-Acetyl-2-thiazoline, 295, 728, 731
$1\alpha,25(OH)_2D_3$, 47–49
α-Amino N, 467
$25(OH)D_3$, 47–48
Absorption, 480
  nutrient, 481
ACE-inhibiting, 110
Acetaldehyde, 104, 660, 667
Acetoin, 271
Acid, 1050
  low-acid canned foods (LACF), 948
  low acid foods, 954
Acid degree value (ADV), 463
Acid index, 235–256
Acidification, 477
Acidified, 480
Acidity, 478
Acidity and pH, 368
Acidophilus, 391, 478
Acidophilus milk, 412
Actin
  aggregation complex of, 878
  carp, 858, 882
  denaturation, 879
  interacting with metal ions, 879
  muscle, 877–878
  thin filament, 878
Actomyosin
  amount of extractable, 878
  ATPase activity of, 878
  changes in muscle, 876
  dissociation of, 878
  during frozen storage, 878
  extractable, 878
  soluble, 878
  viscosity of, 878
Additives, 881, 885
Adenosine dinucleotide phosphate, 381
Advantages, 479
Agglutinin, 385
Aggregation
  breakdown of TMAO, 879
  electron microscopic observation, 878
  formation of, 878
  muscle protein, 878
  myofibrillar protein, 878
Aging time, 476
Agitation, 477
Agricultural business, 450
Alanine, 15, 269–270, 285–286, 289, 465, 508, 579, 905, 936
Alcohol, 478

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

Alkaline phosphatase, 361, 466, 468
Alkalinity, 479
Alkyl-pyrazines, 938
Alleles, 470–471
  specific primers, 471
Allelic
  genotype, 471
  variants, 470
Allergic, 393
Allergies, 166, 450, 454, 479
Allergy, 118
  classification, 118
  definition, 118–119
  mechanism, 115, 119–120
  prevention, 123
  therapy, 126
Alpha lactalbumin, 386
Alpine, 382
Alzheimer disease, 497
Amines, 257, 270, 273, 288
Amino acid, 387, 465, 471
  alanine, 465
  aspartate, 465
  brach-chain (BCAA), 465
  essential (EAA), 465
  glutamate, 465
  glycine, 468
  leucine, 465
  lysine, 465
  proline, 465, 468
  serine, 468
  sulfur, 465
  total, 465
  tyrosine, 465
  valine, 465
Amino acids, 239, 245–246, 262, 268, 272, 283–289, 316, 322
  generation of. *See* Proteolysis
Aminopeptidase N, 723, 725
Aminopeptidases, 239, 245, 270, 272, 287
Ammonia, 268, 287
Ammonium, 731, 737, 905
Amplified, 472
Amplify, 471
Analytical requirements, 1055
Anaphylaxis, 118
Anatomical, 458
Anemic, 481
Angiotensin-I converting enzyme (ACE), 497–498, 501–503, 510
Angora, 454
Antagonism, 452
Antagonistic, 478
Antiaging mechanisms, 136, 138
  anticancer activities, 141
  antioxidant activities, 136
  cardiovascular protection, 140
  eye protection against cataract and macular degeneration (MD), 138
  metal-chelating activities, 138
  neuroprotection, 138
Antibiotic resistance, 93–95
Antibiotics, 166, 457, 650, 660, 665, 667
Anticarcinogenic, 163
Antidiabetic, 176, 179, 181, 185
Antihypertensive peptides, 497
Anti-inflammatory properties, 47
Antimicrobial agents, 885
Antimicrobial compounds. *See* Bacteriocins
Antimicrobial peptides, 503, 505, 509–510
Antimicrobials, 220
Antimicrobial metabolites, 156
Antiosteoporotic and antiobesity effects, 107
Antioxidants, 44–47, 1111
  activity, 60
  application of, 1114
  Chinese medicine, 56
  functional food, 61
  intake, 57
  isoflavones, 58
  olive oil, 62
  plasma, 62
  synthetic, 57
  tomato, 59, 62
Antithrombotic peptides, 496
Aquaculture, 969–979
  cage-culture, 972–973, 975
  extensive, 970
  feeds, 971, 973–974, 976–977
  flesh quality, 975–976, 978
  intensive, 971–979
  polyculture, 969
  pond-culture, 969, 977–978
  production, 970–971
  selective breeding, 975
  semi-intensive, 970
Aroma. *See* Flavor
Aroma extract concentration analysis, 731
Aroma extract dilution analysis, 726–727, 731
Artisanal, 454
Aseptic processing of milk, 333, 339–342
  concept, 339
  equipment design and operation, 339
  extended-shelf-life milk, 341

gelation phenomenon, 342
milk system stability considerations, 342
monitoring of thermal process, 341
packaging platforms, 342
product shelf life, 342–343
the system, 339–341
ultra-high-temperature (UHT) processing, 341
Ash, 381
Asparagus, frozen, 986
styles, 987
cut spears or cuts and tips, 987
spears or stalks, 987
types, 987
green, 990
green-white, 987
Aspartic, 285, 322, 468, 508, 579, 935–936
Atherosclerosis, 468
Atomization, 479
ATP. *See* Nucleotides
ATPase
activity, 870
Autoclave, 478
Autoxidation, 880, 886
Ayrshire, 381

2,3-Butandiol, 719
2,3-Butanedione, 719, 727–732
Bacillus megaterium, 509
Bacteria, 154, 457, 949, 953, 956, 958
Clostridium difficile, (*C. difficile*), 163, 166–167
coliform, 457
count, 457
E. coli, 154
H. pylori, 156
L. acidophulus, 156
lactic acid, 154, 168
bifidobacteria, 154, 163, 166
lactobacilli, 154, 166
streptococci, 154
listeria, 457
Bacteriocin, 268
Bacteriophage, 653, 659
Bacterium lacticus longi (*Lactococus lactis biovar longi*), 416
Bactiocins, 156
heat-laible, 156
lantibiotics, 156
nonlantibiotics, 156
Bags, 869
Balancing ingredients, 602
Batters and breading materials, 920
Beneficial cultures, 102, 348
Beta-galactosidase, 391
Beta-hydroxybutyrate, 383
Beta-lactoglobulin, 386
Bifidobacteria, 101, 446–448, 490, 502
Bioactive peptides, 6, 583
biological effects, 25
eggs, 21
fish, 22
functionality, 46
future applications, 28
meat, 22
milk and dairy products, 18
plants, 24
Bioactive peptides production
enrichment of bioactive peptides, 17
enzymatic hydrolysis, 9
microbial fermentation, 12
Bioavailability, 44, 47–48, 481
carotenoids, 60–62
Biochemical aspects, 857
Biogenic amines. *See* Amines
Biomedical, 454
Blanching, 405, 1110
Blemished, 1051
Blood pressure, 210
Blue cheese, 389
Blue crab, 955
cooking, 955
cooling, 956
packing, 956
pasteurization, 956
pickling, 956
Blue, 499, 501, 502
Blueing, 905, 906
Blue whiting, 932
Boiling point, 377
Bone cells,
osteoblasts, 209
osteoclasts, 209
Bone consolidation, 208
Bone formation, 208
Bone mineral content (BMC or bone mass), 208
Bone mineral density (BMD), 208
Bone resorption, 209

Bone, 44, 48–51, 208
  hip (proximal femur), 208
  lumbar vertebrae, 208
  nonvertebral sites, 208
Bovine, 386
  homolog, 465
  sequence, 471
Bovine serum albumin, 358
Bradykinin, 497, 501–502
Breast milk, 120
  antibody, 120
  cytokine, 120
Breeding
  artificial, 461
  natural, 461
Breeds, 381, 449, 452, 459, 460
Brine, 241, 282
Brix, 1050, 1055
Broccoli, frozen, 988
  styles, 988
    chopped spears or short spears, 988
    cut spears or short spears, 988
    pieces or random cut pieces, 988
    short spears or florets, 988
    spears or stalks, 988
Brown swiss, 381
Brussel sprouts, frozen, 986
Buck, 456
Buffalo, 380–381, 384–385, 391, 451–452
Buffering capacity, 369, 479–480
Buildings, 859
Butter, 474
  dried, 478
Butterfat
  clarified, 478
Buttermilk, 391, 412, 415, 446, 474, 476, 606
  cultured buttermilk, 415
  cultured (fermented), 432, 444
  processing, 443
  traditional natural buttermilk, 415
Butyric acid, 383
Bypas fat, 383
By-product, 478

α_{s1}-CN, 491, 493, 495
β-Carotene, 42, 44, 46, 469
β-CN, 491, 493, 494
Caciocavallo, 509
Cadaverine. *See* Amines
Cajeta, 478
Calcifications, soft-tissue, 202, 210
Calcified (mineralized) plaque, arteries, 210
Calcitriol (vitamin D metabolite; hormone), 205
Calcium, 386, 388, 394
  from foods, both natural and fortified, 202
  from supplements, 202
  sequestrant, 474
Calcium, blood or serum, 209
  parathyroid hormone, 209
  regulation, 209
Calcium, functional role, 105–107
Calcium-sensing receptor (CaSR), 205
Callinectes sapidus, 897
Calpains. *See* Proteinases
Calpis, 497, 501, 503
CAM, 177–178
Camel, 385
Camembert, 502
Cancer, 469
  breast, 206
  colon and rectal, 203
  ovarian, 206
  prostate, 205
Cancer magister, 897
Canestrato Pugliese, 505–506, 509
Canned tomatoes, 1096–1099
  additives, 1098
  blanching, 1098
  firming agents, 1099
  pectinmethylesterase, 1098
  pectinpolygalacturonase, 1098
  peeling, 1097
  quality standards, 1099
  sorting and grading, 1096
  thermal processing, 1099
Canned Tuna, 953
Capelin, 932
Cappuccino, 404–406
Capric acid, 383
Caprine, 387
Caprino del Piemonte, 505–506, 509
Caproic acid, 383, 732
Caprylic acid, 383
Captopril, 502–503
Caramel, 404–406
Carbohydrates, 101, 105, 257, 266, 284–285, 287–288, 468, 476, 688
Carbonation, 406
Carbon dioxide, 478
Carbonyl sulfide, 724

Cardiac function, 469
Cardiovascular diseases, 46
Carnitine, 458
Carotenoids, 43–44, 56, 58, 61, 976, 1112
  bioavailability, 60
  β-carotene, 61
  tomato, 59
Carrageenan, 404–405, 605
Carrots, frozen, 988–989
  styles, 988
    chips, 989
    cut, 989
    halves, 989
    quarters, 989
    sliced, 989
    strips, 989
    wholes, 989
Case hardening, 1113
Casein, 380, 386–388, 391–393, 558–559
  acid casein, 567
  applications, 570
  associative state, 560
  casein co-precipitates, 564
  casein fractions, 560, 569
  casein micelle, 570
  caseinates, 569
  exports and imports, 560
  global production, 558
  hydrolysates, 562
  micellar casein, 568
  molecular properties, 559
  rennet casein, 564
  stabilizer interaction, 653
  yogurt gel strength, 652, 660, 664
Casein, bioactive peptides derived from, 109
Caseinoglycopeptide, 49
Caseinomacropeptide, 504
Caseinophosphopeptides, 493–495, 510
Caseins (CN), 464, 471, 695, 705
  α-, 464
  αs1-, 464, 471, 472
  αs2-, 464
  β-, 464
  κ-, 464
  alleles, 472
  genotype, 474
  loci, 464
  micelles, 472
  polymorphisms, 458
Casing, 252, 257–258, 261, 304
Casocidin, 500, 504–505
Casokinins 490, 495, 497–499, 502, 509
Casomorphins, 490–493, 495–496, 499, 503, 509
Casopiastrin, 496
Casoplatelin, 496, 509
Catalase, 267–268
Catalytic
  reaction, 465
Catfish, North American freshwater, 849–851
  bone classifications, 850
  definitions, 851
  description, 849
  market forms, 850
  styles, 850
  types, 850
Cathepsins, 239, 723, 756
Cauliflower, frozen, 989
  styles, 989
    clusters, 989
    nuggets, 989
Cell differentiation, 49
Cell envelope-associated proteinases, 723
Cell proliferation, 44–48
Cell signalling, 44, 46, 49
CEP. *See* Cell envelope-associated proteinases
CGMP, 950, 952
CGMPR, 950
Cheddar, 394, 499, 501, 503
Cheese, 387, 391, 450, 476
  brown whey, 478
  cheddar, 464
  yield, 470, 472
Chemical
  characteristics, 461
  composition, 449
    general, 458
  structure, 461
Chemical anchors for specific flavors or off-flavors, 733
Chemical composition, 858
Chemical composition of milk, 348
Cheshire, 719
Chhana, 478
Chilling, 478
Chinonectes spp., 897
Chitin, 908
Chitosan, 908
Chloride, 731
Chloride salts, 920

Chocolate, 404–409, 617, 640, 641
Chocolate mix, 479
Cholecalciferol, 47
Cholesterol, 273, 461
  deposition, 480
  dissolution, 480
  esterified, 463
  esters, 462–463
  free state, 461
  metabolism, 480
Chronic enteropathy, 479
Churning butter, 478
Chylomicron, 383
Chyluria, 480
Chymosin, 495–496, 504, 506–507, 723–724
Chymotripsin, 494, 502
Cis-4-heptenal, 938
Citrate (or citric acid), 388, 637, 644
Citrate metabolism, 719
Citric acid, 404–405, 462
Clams
  hard clam (quahog), 898
  processing, 905
  sea (skimmer) clam, 895
  soft clam, 898
  surf clam, 898
Clarification, 478
Climate, 452
Clostridium botulinum, 341
Clotting factors, 49
Coagulating ability, 472
Coagulation, 477
  properties, 470
  slow, 476
Coating(s), 639, 641
Cocoa, 404–405, 479, 637
Cod, 858, 874–876, 878–879, 884–885, 932, 935, 942
Code of federal regulations, 595
Codex Alimentarius Commission, 1009–1010
  HACCP, 1009
  quality, 1010
  retail, 1010
  safety, 1010
Coefficient α, 861, 862
Coffee, 404, 408
Cold shortening problem, 877
Coliform bacteria, 457
Colligative properties, 376
Color, 237, 244–245, 252, 256, 261–262, 267, 308, 315, 366, 1051, 1055, 1061
Comminution, 258
Commonality, 465
Competition for substrates, 155
Complementary and alternative medicine (CAM), 177
Composition, 449, 459
Composition of milk, 348
Compressor, 872
Comte, 739
Concentrate, 456, 479
  diet, 459
  feed, 456
Concomitantly, 461
Condensed milk, 602
  superheated, 603
Condensed products, 474
Condenser, 479
Conditioned, 1015
Conditioning, 1015–1016
Conformation, 465
Conjugated linoleic acid (CLA), 106, 383, 463
Consistency, 477
Constituent, 467
Consumer acceptance, 882
Consumer taste, 450
Consumption, 451, 454
Containers, 859, 869
Contaminants, 953, 959
Contamination, 859, 868, 870, 873, 880–881, 887
Convalescent treatment, 580
Cooked ham, 241, 249, 286, 291, 296, 312–315
  cooling, 244
  massaging, 243
  reception of hams, 241–242
  resting, 243
  smoking, 244
  tumbling, 243
Cooperative, 454
Copper, 390, 458
Corn on the cob, frozen, 989–980
  colors, 990
  lengths, 990
  styles, 989
    natural, 989
    trimmed, 989
Corn syrup, 479, 603
Coronary bypass, 480
Cottage cheese, 334
Cottage industry, 476

Cow breed,
Holstein, 460, 480
Jersey, 460, 480
Cow, 380, 383–387, 389–391, 393–395
Cowy flavor, 733
Coxiella burnetti, 336
Crab, 882–883, 932–942
Crabs
Alaska king crab, 897
blue crab, 897
blueing, 905
brabmeat processing, 897
Dungeness crab, 897
mushiness, 905
stone crab, 897
swimming crab, 897
Tanner crab, 897
walking crab, 897
Cragon franciscorum augustimana, 895
Crassostrea
gigas, 898
virginica, 898
Creatin, 467
Creatinine, 467
Crescenza, 499, 501, 503, 506, 509
Critical control points, 948
Crossbreeding, 454
Cross-linkages
intermolecular, 879
Crustaceans, 932–942
Crystal(s), 394, 637–638
ice crystals, 637–641
Crystallization
ice, 860, 862, 864
Cucumbers, 1046
Cultured cream, 415, 444–446
Cultures, 476
probiotic, 102
yogurt, 102
Curd, 391
Curd tension, 375, 475, 477
Curing salt, 236, 252
Current good manufacturing practices (CGMP), 950
Current good manufacturing practices regulations (CGMPR), 950
Cystic fibrosis, 480
Cytomodulatory peptides, 495
Cytoplasm, 382

2,3-Decadienal, 940
7-Dehydrocholesterol, 47–48
Dairy, 450–451
cows, 451
goats, 451–452
farming, 450
industry, 450, 454
products, 450
sheep, 450
farming, 450
species, 450, 456
De novo, 383
Decarboxylation, 268, 270, 288–289
Decomposition, 949–952, 954, 959–960
Decreamed, 478
Defects, 1055
Deficiency, 40–41
vitamin A, 44
vitamin D, 47–49
vitamin E, 45–46
vitamin K, 50–51
Definition of milk, 349
Degrees Baumé, 1050–1055
Dehydration, 823–826, 830, 859, 866–868, 887
osmotic, 1114
tomato, 1110
Deletion, 470
Denaturation
freeze, 888
protein, 857–858, 877–880, 883
Density, 373
Depression, 459
Descriptive sensory analysis, 731
Desertification, 452
Destabilization,
Detoxify, 467
Dextrose equivalent, 479
DFD meat, 235, 241, 322
DHDA. *See* Dynamic headspace dilution analysis
Diabetes, 177–179, 185, 188–189
Diameter, 461
Diced tomatoes, 1100
quality standards, 1100
size reduction, 1100
Diet, 468–469
low-fat, 478
Dietary approaches to stopping hypertension (DASH), 210
Dietary manipulation, 463
Digestibility, 479
Dimethylamine (DMA), 858, 877, 881, 884, 942
Dimethyl disulfide, 724, 731, 941

Dimethyl sulfide, 724
Dimethyl tetrasulfide, 724
Dimethyl trisulfide, 724, 941
Dipeptidylpeptidases. *See* Peptidases
Discoloration, 824–825, 827, 829, 845
Distribution, 480
Disulfide bonds, 465
DMA. *See* Dimethyl amine
DMDS. *See* Dimethyl disulfide
DMS. *See* Dimethyl sulfide
DMTS. *See* Dimethyl trisulfide
DNA, 470
  fragment, 472
  rearrangement, 472
Drained weight/volume, 1054
Draining, 477
Drier
  solar, 1115
  tunnel, 1116
Drip, 857, 864, 876–878, 882–883, 906
Dry-cured ham, 233–249, 282, 285–286, 290–294, 304, 316–323
  accelerated processing, 241
  boned, 237
  drying, 237, 322
  post-salting, 234, 237
  pre-salting, 236
  reception of hams, 234–236
  ripening, 237
  smoking, 237
  traditional processing, 234, 241
  types of
    Bayonne, 240
    Corsican, 240, 323
    country-style, 236, 241, 323
    French, 222, 236, 240
    Iberian, 237, 240, 292, 323
    Italian, 240
    Katenschinken, 241
    Kentucky, 241
    Parma, 236, 240, 292
    San Danielle, 240
    Serrano, 323
    Spanish, 236, 240, 292
    Virginia, 241
    Westphalia, 241
    Yin Hua, 241
    Yunnan, 241
Drying, 404, 406, 477
  convective, 1116
  roller, 479
  spray, 477, 479, 1117–1118
Dynamic headspace dilution analysis, 731
(E)-4,5-epoxy-(E)-2-decenal, 726
2-Ethyl-3,5-dimethylpyrazine, 731
4-Ethyl octanoic acid, 731
4-Ethyloctanoate, 463–464
Economy, 450
  national, 450
Eczema, 166
Edam, 502
Edible films and coatings, 799
  active packaging, 807–808
  antimicrobial agents, 799–808
  antimicrobial packaging, 808
  antioxidants, 807–808
  applications, 800–804
  carbon dioxide generating systems, 807
  composite films, 804
  definition, 799
  ethanol generating systems, 807
  formation mechanisms, 805
    complex coacervation, 805
    gelation, 805
    simple coacervation, 805
  history, 799
  importance, 799–800
  lipid based, 800–801
    glycerides, 801
    waxes, 801
  manufacture, 805
  methods for application, 806
  plasticizers, 805
  polysaccharide based, 801
    agar, 802
    alginates, 801–802
    carrageenan, 802
    cellulose and cellulose derivates, 803
    chitosan, 802–803
    starch, 802
  protein based, 803–804
    corn zein, 803–804
    gelatin, 803
    soy protein isolate, 804
    whey protein isolate, 804
Effectiveness, 468
Effects of heat, 371
Egg products, 606
Egg yolk solids, 479
Eland, 380
Electrical conductivity, 370
Electrochemical properties, 370
Electrophoresis, 470
Electrophoretic
  mobility, 465

Embden–Meyerhof–Parnas, 101
Emmental, 502
Emulsifier(s), 479, 596, 604, 606, 643
Enalapril, 501
Encephalopathy, 168
Endonucleases, 471
Endopeptidases, 933–935
Energy, 454, 480, 873, 875–877
Enkephalins, 491, 497
Enterocins, 91, 93
Enterococcus faecium, 509
Enterococcus spp., 86, 91–92, 94
  Enterococcus faecalis, 86
  Enterococcus faecium, 86
Enthalpy, 861
Environment
  conditions, 459
  tropical, 460
Enzymatic reactions, 239–240, 245–246
Enzyme inactivation, 220, 243
Enzymes, 387, 467, 471
  alkaline phosphatase, 468
  glutathione peroxidase, 469
  lactic dehydrogenase, 468
  lipase, 463
  lysozyme, 467
  malic dehydrogenase, 468
  protease, 467
  ribonuclease, 467
  xanthine oxidase, 468
Epilepsy, 480
Epithelial cell, 389
Epithelial surface, 156
  competition, 156–157
    adherence, 157
    receptor, 156
Equalization, 1050
Ergosterol, 47–48
Erythritol, 639
Escerichia coli, 490, 504–505, 507–509
Ester linkage, 480
Esterases. *See* Lipases
Ethyl furaneol, 731
Evaporated, 474
Evaporation, 479
  chamber, 479
Ewe, 476
Exonic point deletion, 470
Exopolysaccharide, 654, 660, 666
Exopolysaccharides (EPS), 416, 440
Exorphins, 491–492
Exotic types, 476
Exopeptidases, 932–934
Extraneous vegetable material (EVM), 1052
Extruded (also extrusion, and cold extrusion), 641–644

Fabrication, 476
Factors, 452
FAO, 450–451, 465
  WHO, 465
  yearbook, 450
Farmstead, 477
Farmstead cheese makers, 454
Fat, 381, 385, 389, 456, 637, 642–643
  globule, 458, 461
    size, 460–461
Fatty acid, 239–241, 245–246, 256, 262, 272–273, 283–285, 308, 380–383, 447, 461, 463, 818, 877–878, 880, 886
  branched-chain (BCFA), 463
  capric, 463
  caproic, 463
  caprylic, 463
  composition, 443, 464
  essential, 465
  lauric, 463
  medium chain (mct), 463, 480
  methyl esters, 463
  monomethylated, 463
  oleic, 463
  palmitic, 463, 480
  short chain, 464, 472
  unsaturated, 472
  volatile, 472
    insoluble, 472
    soluble, 472
Fatty acids metabolism, 722
FDA, 948–950, 953–954
Feeding, 452
  supplementary, 454
  under, 454
Feed off-flavors, 733
Fermentation, 96, 98, 100, 102, 260, 265–274, 478
  alcoholic, 478
Fermented, 478
  products, 474
Fermented milks, 411
  claims, 100
  classification, 412–413, 432
  definition, 103

Fermented milks (*Continued*)
fermented milks with yeast and lactic starter cultures, 423–425
health benefits, 106
antiosteoporotic and antiobesity effects, 103
lactose intolerance, 102
physiological activity, 104
manufacturing, 413–426
nutritional aspects, 101
patterns of production in europe, 413
process flowchart, 440
production, 97
regulatory aspects, 99
requirements for production, 434
milk homogenization, 437
milk pasteurization, 436
milk quality, 434
quality and functionality of other components, 434
types of culture, 434, 437
set-type products, 416
shelf life of product, 441
stirred-type products, 415
trends in asia, 433
use of mesophilic bacterial strains, 415–417
use of probiotic bacterial strains, 422–425
use of thermophilic bacterial strains, 417–422
weight loss, 104
Fermented sausages. *See* Sausages
Fibrinogen, 496
Fill of container, 1053
Fillet, 857, 861–864, 867, 870, 874–876
Film-jolk, 432
Filtering, 477
Filth, 947, 949, 955, 958, 1058, 1060–1061
Firmness, 472–473
curd, 472
Fish
Asian seabass, 972, 978
Atlantic salmon, 970, 975
blood, 882
carp, 970, 971
Channel catfish, 970, 971
composition, 858, 877, 885
discoloration, 882
fatty, 858, 877, 884–885
fillet, 861–864, 876
flavor of, 883–884
fresh, 856–857, 881, 883, 885
frozen, 857, 874, 878–879, 881, 887
glycogen content, 877
health, 972–973
lean, 858, 885
mackerel, 857, 885–887
mince, 879, 883, 887
moisture, 863
muscle, 857, 859, 877–880, 883
number of myotomes, 858
nutrition, 973–974
odor of, 883
oxidation of, 882
property of, 878
protein, 883
quality, 856
rainbow trout, 970
size of, 858
species, 857–858, 877, 884–887
surface, 875, 882
texture, 883
tissue, 857, 879, 881, 885, 888
tuna, 977–978
types of skeletal muscle, 858
value-added, 857
white, 373
white muscle, 358
Fish and mince meat quality, 917
Fish meal, 973
Fish oil, 973–974
Fish, formulated, 916
product thawing during retailing and home preparation, 926
quality control during the processing, 920
quality parameters of the formulated products, 916
shelf-life, 926
Fish, processed, 916
Fish protein hydrolysates, 932–933
Flanking, 472
Flavonoids, 56–57, 59
degradation, 58
fruits, 58
tomato, 58
Flavor, 237, 239–241, 244, 246, 252, 257, 261, 268, 271–273, 282, 285–296, 304–305, 312–323, 367, 474, 874, 881, 883–884, 887–888
alteration, 474
damage, 479
ingredients, 476
Flavor and odor, 831, 1052, 1055
Flavoring, 257, 260

Flavorings produced by enzymatic hydrolysis, 931–940
Flavors, milk and mik products, 716
  characterization, 726
  chemistry, 733
  production, 716
  taste compounds, 736
Flavors, seafood byproducts, 932
Fluctuation, 468, 870–881, 885
Fluid milk, 599
Foaming, 373, 404–405
Folate (folic acid), 154, 205, 467, 470
  binding protein, 467
Food alterations, 229
Food and Drug Administration (FDA), 986
  approved health claims,
Food and Nutrition Board, 208, 211
Food Code, 950
Food for special health use, 446
Formaldehyde (FA), 858
Formulation of ice cream, 597
Fortified, 474
Fossa, 509
FPH. *See* Fish protein hydrolysates
Fractures, skeletal, 202, 209
Free amino acids, 858
Free fatty acid (FFA), 463–464
Freezer, 861–864, 868, 875
Freezing (and freezing point), 638–639, 641
  apparatus, 861
  biochemical aspects of, 857
  conditions, 881
  damage, 864, 885
  effect on quality, 859, 881
  equipment, 859–860, 863–866, 868, 873, 875
  medium, 867
  method, 863–864, 867
  physical point of view, 862
  physicochemical change, 876–877
  point, 860–863
  preservation, 857
  process, 861–862, 881
  rate, 860, 862–863, 865
  ratio, 863
  seafood, 877
  speed, 862, 864
  temperature, 860–861
  time, 860–862, 864
Freezing methods
  blast freezing, 899–900
  carbon dioxide freezing, 900–901
  cryogenic freezing, 900–901
  immersion freezing, 901–902
  liquid nitrogen freezing, 900
  plate freezing, 899
  pressure shift freezing, 902
Freezing point, 376
Freezing point depression, 604
Freezing process, 924
French vanilla, 479
Fresh and frozen shrimp, 840–842
  examination, 841
  market forms, 841
  styles, 840–841
  types, 840
Fresh cream, 600
Friable, 480
Frost, 863, 865, 867, 871–873, 875
Frozen
  products, 474
  yogurt, 474
Frozen avocado halves, 1008
  cold chain, 1008
  IQF belt freezer, 1008
Frozen avocado paste and guacamole, 1003–1009
  avocado paste, 1005
  blast freezer, 1007
  cryogenic freezer, 1007
  freezing, 1006
  frozen storage, 1007
  guacamole, 1006
  liquid nitrogen, 1007
  packaging, 1006
  quality standard, 1007
  sorting and grading, 1004
Frozen avocados, 997–1011
  biology, 999
  climateric, 1000
  Hass avocado, 1001
  nutritional value, 1003
  oil, 999
Frozen cream, 600
Frozen desserts, 593
  classification, 595
  composition, 597
  federal standards, 596
  formulation, 597
  mix calculation, 606
  trade classification, 596
  trends in market, 596

Frozen fish fillet blocks, 826–829
  defects, 826
    belly flaps, 827
    blood spots, 827
    bones, 828
    bruises, 827
    dehydration, 826
    discoloration, 827
    fins, 828
    foreign material, 828
    improper fill, 827
    membrane, 828
    parasites, 828
    scales, 828
    skin, 827
    texture, 828
    underweight, 826
    viscera, 827
Frozen fish, whiting, 822–823
  grading, 822
    bruises, 823
    color, 823
    dehydration, 823
    evisceration, 823
    flavor and odor, 823
    heading, 823
    minimum size, 823
    scaling, 823
    texture, 823
Frozen foods, 857, 859–860, 862–863, 868–870, 873, 876, 880–881
Frozen fried fish portions, 839–840
  composition, 839
  examination, 840
Frozen fried fish sticks, 837–840
  composition, 836
  examination, 836
Frozen halibut steaks, 823–824
  defects, 824
    dehydration, 824
    discoloration, 824
    honeycombing, 824
    non-uniformity, 824
    texture, 824
    workmanship, 824
  styles, 823
    random weight, 823
    uniform weight, 823
Frozen ham, 236
Frozen headless dressed whiting, 822–823
Frozen minced fish blocks, 829–832
  color classifications, 829
  defects, 829
    angles, 830
    blemishes, 831
    bones, 831
    dehydration, 830
    discoloration, 829
    flavor and odor, 831
    improper fill, 830
    texture, 829
    uniformity of size, 830
    uniformity of weight, 830
  texture, 829
  types, 829
Frozen raw breaded fish portions, 835–837
  composition, 836
  examination, 836
  styles, 836
Frozen raw breaded fish sticks, 833–835
  composition, 834
  definitions, 834
  examination, 834
Frozen raw breaded scallops and frozen fried scallops, 847–849
  definitions, 848
  description, 847
  styles, 848
  texture, 849
  types, 848
  workmanship defects, 848
Frozen raw breaded shrimp, 843–846
  styles, 843
Frozen raw fish portions, 832–833
  defects, 832
    blemishes, 832
    bones, 833
    dehydration, 832
    texture defects, 833
    uniformity of shape, 832
    uniformity of size, 832
  styles, 832
  types, 832
Frozen raw scallops, 846–847
  definitions, 847
  styles, 846–847
  types, 847
Frozen salmon steaks, 824–826
  defects, 826
    discoloration, 826
    honeycombing, 826
    non-uniformity, 826
    texture, 826
  dimensions, 826

species, 824
styles, 825
random weight, 825
random weight combination, 825
uniform weight, 825
Frozen storage and retailing, 924
Fructose, 639
Fruit, 404, 406
banana, 404–406
date, 405
diet, 57, 58
flavonoids, 58
grape, 404
mango, 405–407
orange, 404–406
raspberry, 404, 406
strawberry, 404, 406–407
Functional food, 60, 120, 154, 446
fish oil, 116, 120, 128
DHA, 116, 120, 128
EPA, 116, 120, 128
inflammation, 120
probiotic, 120, 124, 127
Functional foods and herbs, 141
broccolis, 141, 143
calcium, 142
cocoa and chocolate, 142
coffees (*Coffea arabica*), 144
curcumin (diferuloylmethane), 143
fish, fish oil, and olive oil, 141
fruit and vegetable fibers, 143
garlic, 143–144
grapes and wine, 144
propolis, 143
soy foods, 142
teas (*Camellia sinensis*), 144
tomato, 138, 141
vitamin A, 138
Functional properties, 40, 42, 44, 46, 48, 50

Galactose, 391
Galactosaemia, 582
Gallstones, 480
Gangliosides 686
Gas chromatography olfactometry/aroma extract dilution analysis, 731
Gastrointestinal, 163, 450
allergy, 479
disorders, 450, 454
GCO/AEDA. *See* Gas chromatography olfactometry/aroma extract dilution analysis
GCO-H. *See* Static headspace-olfactometry
Gelatin, 605
Gelation, 387
Gelato, 595
Gene
coding, 471
frequency, 470
Generally recognized as safe (GRAS), 202
Genetic, 452
polymorphism, 464, 472
rearrangement, 472
selection, 452
typing, 472
Genotype, 471
Geotrichum candidum, 412, 425
Ghee, 478
Ginseng, 171–172, 174–182, 184–193
Ginsinoside(s), 176–179, 181, 184, 186–193
Gjetost, 478
Glazes, 887
Glucose. *See* Carbohydrates
Glucose, 465, 639
Glutamic acid, 887
Glutathione peroxidase, 469
Glyceride, 472
Glycerin, 639
Glycerol, 458, 472
ethers, 458
Glycine, 936
Glycine betaine, 905
Glycolysis, 271, 284, 877
Glycolysis and related reactions, 719
GMP, 949
GMPR, 950, 954
Goat, 380–381, 383–387, 389–391, 393–395, 477
keepers, 454
pedigreed, 454
population, 451
Goat breeders, 454
Goat breeds, 459
alpine, 452, 467–468, 480
Anglo-Nubian, 452, 460, 468
Canaria, 452
Damascus, 452
French-Alpine, 468
garganica, 471
Indian jamunapari, 452
indigenous, 460
African Dwarf, 460
Red Sokoto, 460
La Mancha, 460

Goat breeds (*Continued*)
Malaguena, 452, 471
Maltese, 452, 471
Murciana-Granadina, 452, 471
Nubian, 460, 480
Oberhasli, 452, 460
Poitevine, 471
Saanen, 452, 460, 470–471
Swiss, 460
Toggenburg, 452, 460
Goat cheese, 454
Goat milk, 450–451, 454, 456
anemia, 481
cheese, 454
artisanal, 454
Chabicou, 476
Chabis, 476
Crottin du Chavignol, 476
Les Pyramides, 476
Sainte Maure, 476
cheesemaking, 456
chemical composition, 486
fluid, 474
production, 450–451
economics, 456
products, 478
cosmetic, 478
evaporated, 474
fermented, 477
frozen, 454
powdered, 474
uniqueness, 458
yogurt, 477
Goaty flavor, 463
Good manufacturing practices (GMP), 343, 949
clean room processing environment, 343
compliance with public health regulations, 344–345
employee training, 343
written standard operating procedures, 343
Gorgonzola, 501, 509
Gouda, 496, 498, 501–502
Grade A milk, 389
Grade A pasteurized milk ordinance (PMO), 332–333
Grade(s), 1055–1056
Greenland halibut, 932
Grit, 1057, 1061, 1062
Growth parameters, 481
Guaiacol, 726
Guar gum, 605
Guernsey, 381
Guinea pig, 393
Gums 404–405

2,4-Heptadienal, 940
3-Hydroxy-2-butanone, 719
α-Hydroxy acids, 725
HACCP, 948, 950
Haddock, 932
Ham. *See* Cooked ham, Dry-cured ham
Hand lotion, 478
Haplotype, 471
Havarti, 502
Hay, 456
Hazard analysis critical control point (HACCP), 343, 909, 946, 948
frozen shellfish, 909
Health, 463
food store, 457
Health claim, 70
Heat, 218, 220, 473
coagulation, 474
coefficients, 220
cheese, 218
meats, 220
conductivity, 219–220
desiccated, 478
exchangers, 479
heat transfer, 222
penetration, 228
stability, 473
Heat capacity, 370
Heat shock, ice cream, 628–629
Heat stability, 372
Hemoglobin, 470, 479
iron, 481
regeneration efficiency, 481
Herbicide, 474
Herbs, 176, 178
Herds, 459
Heritable, 471
Hexanoic acid, 726
Hinterland, 454
Histamine. *See* Amines
p-HPEA and DPHEA, 59
Holstein, 381
Homeostasis, 48
Homerus americanus, 896
Homofuraneol, 732
Homogenized, 384
milk, 461
Homology, 470

Homozygous, 472
Hormone, 47, 49
Horse, 380
Human, 385, 392
  milk, 467
  nutrition 458
Hydration, 472
  degree of, 473
Hydrocolloids, 605, 637–638
Hydrocolloids and nonfish proteins, 918
Hydrolysates, 698, 705
Hydrolysis, 491, 493–496, 501, 503, 509
Hygiene, 859, 860
Hypercholesterolaemia, 168
Hyperglycemia, 176–177
Hyperlipoproteinemia, 480
Hypersensitivity, 118–119
  classification, 118
Hyperuricaemia, 582
Hypocholesterolemic, 480
Hypocholesterolemic peptides, 496–497
Hypoxanthine, 858, 907

2-Isobutyl-3-methoxypyrazine, 733
Ice, 857, 860–864, 873, 876, 881–883, 886–887
Ice cream, 594
  formulation, 597
  market trends, 596–597
  nutritional value, 630, 632
  premium, 596
  role of components, 600
  shelf life, 626
Ice cream freezers, types, 622
  continuous, 622–623
Ice cream novelties, 630
  extruded novelties, 632–633
  molded novelties, 630–631
Immune, 41, 43–45, 49
Immunity, 157
Immunoglobulins, 358, 384, 465
Immunological
  cross-reaction, 479
Immunomodulator, 157
Immunomodulatory peptides 495, 510
Inactivated, 468
Incentive, 457
  payment, 457
Incidental, 457
  sale, 457
Incubation, 477–478
Indole, 905, 909
Industrial process, 58–59, 62
Infant formula,
  soy-based, 481
Infant formulas
  carbohydrates, 688
  functional compounds, future, 706
  intestinal microflora, 689
  lipids, 679
  long chain polyunsaturated fatty acids (LCPUFA), 680
  oligosaccharides, 690, 692
  prebiotic factor, 690, 692
  preformed dietary LCPUFA as components of biological membranes, 680
  recommendations for the supplementation of infant formulas, 685, 692
Infant nutrition, 579
Infection, 155
Inflammation, 155
Inflammatory bowel disease, 161, 166
Inoculation, 477
Inosine, 858
Insect(s), 950–951, 953–955, 957, 1056–1057, 1059
Insecticide, 474
Insertions, 472
Institute of Medicine (IOM),
  Food and Nutrition Board, 208, 211, 212
Intensity, 464
Intestinal resection, 480
Intolerance, 392
Inverse relationship, 460, 468, 473
Inversions, 472
Iodine index, 235, 256
Iodine, 458
  values, 472
Ionizable group, 465
Iron, 390, 458
Irritable bowel syndrome, 163
Isoelectric point, 877
Isoflavones, 57, 394
Isolation, 465
Isracidin, 490, 504–505, 509
Italico, 501

Jarlsberg, 499, 501, 503
Jersey, 381
Juices, 636, 869

β-Ketoacids, 720, 722
Kappacin, 500, 504, 506, 509

Kashk, 478
Kefir, 100–101, 412, 423, 432, 478, 661
  grains, 478
  kefir grain, 423–424
  microflora, 423
  processing, 443–444
Khoa, 478
Kidding, 460
Kidneys, 210
Klebsiella pneumoniae, 495
k_m, 465
Kneading, 478
Koumiss, 412, 423
  microflora, 423
Kumys, 102
K-value, 909

α-Lactalbumin, 357, 464
β-Lactoglobulin, 357, 464
L. reuteri, 446
Labor cost, 456
LACF, 948
Lactaid, 391
Lactalbumin, 491–493, 500, 506
Lactase, 391
Lactation, 456
Lactenin, 504–505
Lactic acid, 257, 266, 268, 271, 284, 315, 390, 478
Lactic acid bacteria, 88–89, 97–99, 491, 503, 510, 718
  non starter, 89
  viable, active, 99
Lactic acid bacterium function, 446–447
Lactitol, 639
Lactobacillus
  acidophilus, 478
  bulgaricus, 477
Lactobacillus acidophilus, 96, 98, 446–447, 500, 502, 652, 664, 667–668
Lactobacillus bulgaricus, 96, 98–99, 647, 650, 653, 657, 662, 667
Lactobacillus casei, 501
Lactobacillus delbrueckii subsp. *bulgaricus*, 501
Lactobacillus helveticus, 494, 497–499, 501, 506, 509
Lactococcus lactis, 446
Lactococcus lactis subsp. *cremoris*, 501, 503
Lactococcus lactis subsp. *lactis*, 502–503
Lactococcus lactis, cremoris, 99
Lactoferricin, 490, 492–493, 500, 504, 507–509
Lactoferrin, 358, 465, 490, 492–493, 503–504, 507–509
Lactoglobulin, 491–493, 496, 500, 502, 506–507, 509
Lactokinins, 497, 500
Lactoperoxidase, 360, 388
Lactorphins, 491, 493, 500
Lactose, 362, 381, 385, 390–391, 458–459, 466, 639
  crystals, 478
Lactose intolerance, 166, 581, 669
Lactose intolerance, alleviation, 105–106
La Mancha, 382
Large intestines, 478
Layout, 870, 871
Lb. bulgaricus, 438
Leafy greens, frozen, 990
  styles, 990
    chopped, 990
    leaf, 990
    pureed, 990
  types, 990
    beet, 990
    collards, 990
    dandelion, 990
    endive, 990
    kale, 990
    mustard, 990
    spinach, 990
    Swiss chard, 990
    turnip, 990
Length, 1052
Leu. citrovorum, 437, 439, 444
Leuconostoc mesenteroides, 502
Leuconostoc, 101, 103
Light activated off flavor, 655, 660
Light ice cream, 595
Lighting, 872
Lima beans, frozen, 987
  lumin, 154
  types, 987
    fresh-seed, 987
    thick-seeded, 987
    thin-seeded, 987
Linoleic acid, 383
Lipase, 383, 387–388, 392, 463, 468
  activity, 468
  lipoprotein, 468
Lipase, milk, 648, 649, 652
Lipases, 239–240, 245–246, 256, 268, 272, 283

Lipid
  hydrolysis, 880, 884
  oxidation, 882, 884, 886–888
Lipid autoxidation, 858
Lipid oxidation, 240, 272–273, 717, 925
Lipids, 239–241, 245–246, 256, 262, 272, 273, 283–285, 289–290, 305, 458, 461
  bound, 461
    glyco, 461
    neutral, 461
    phospho, 461
    polar, 461
  free, 461
    diglycerides, 461
    monoglycerides, 461
    triglycerides, 461
  goat milk, 461
  protected, 459
Lipolysis, 239–240, 245–246, 262, 272, 283–284, 322, 383, 457, 463
  spontaneous, 463, 468
Lipolysis and related reactions, 719
Liquid sugar, 602
Listeria, 457
Listeria innocua, 509
Listeria monocytogenes, 504
Liver necrosis, 469
Lobster, 882–883
  American lobster, 897
  blueing, 905
  freezing and packaging requirements, 903–904
  mushiness, 906
  rock/spiny lobster, 896
Locality, 459, 476
Locust bean gum, 605
Lutein, 61
Lycopene, 1111–1112, 1115
  heating, 59–60
  plasma, 60–62
  supplementation, 62
Lymphocytes, 389, 495
Lysozyme, 388, 467

2-Methyl-1,4-naphthoquinone, 49
2-Methyl-3-phytyl-1,4-naphthoquinone, 49
2-Methyl propanal, 939
3-(Methylthio)-propanal, 938
6-Methyl-hexadecanoate, 463
4-Methyoctanoic acid, 464
4-Methyl octanoic acid, 731
Machine, 869
Mackerel, 857, 885–886
  fillets, 887
Macrobrachium rosenbergii, 895
Magnesium, 458
Maillard reactions, 245–246, 286–289, 296
Malabsorption, 480
Malnutrition, 451
Maltitol, 639
Maltodextrins, 603
Mammary gland, 382
Management system, 456
Manchego, 499, 501
Manufacturing, 473
  procedures, 476
  techniques, 476
  technology, 477
Marsupial, 385
Mass transfer, 222
Mastitis, 389
Meat products
  bacon, 306
  cooked. *See* Cooked ham
  fermented sausages. *See* Sausages
  frankfurters, 308–312
  ham. *See* Dry-cured ham
  roast beef, 305–308
Mechanical damage, 1053
Mechanically deboned poultry meat, 776–780
  color, 776
  composition, 776
  functional properties, 777
  microbiological properties, 777
  nutritional properties, 776
  process, 776
  sausages manufacture, 778–779
    gelling polysaccharides, 779
    textural profile analysis, 779
  surimi process, 778
Medical, 450
  afflictions, 450
  needs, 451
Mediterranean region, 450
Melanosis, 882, 905
Mellorine, 595
Menaquinone-7, 49
Menippe mercenaria, 897
Meta-analysis, 190–191, 204
Metabolic, 161, 458
Metabolism, 44, 46, 49–50, 458, 877
Metal drum, 479
Metallic, 390
Methanethiol, 720, 722, 729

Methyl ketones, 722, 731
Micellar, 472
  characteristics, 474
  hydration, 473
  mineralization, 473
  size, 472
Micelle, 383
Micelle structure, 473
Microbiology, 864
Microorganisms, 218, 226, 857. *See also* Starter cultures
  Alternaria, 218
  Bacillus coagulans, 219
  Bacillus macerans, 219
  Bacillus polymyxa, 219
  Clostridium botulinum, 219
  Coxiella burnetti, 226
  Candida. *See* Yeast
  Clostridium. *See* Pathogens
  Debaryomices. *See* Yeast
  Enterobacteriaceae, 905
  Escherichia coli. *See* Pathogens
  Fusarium, 218
  Kocuria, 266–268, 272
  lactic acid bacteria, 253, 266–270, 284, 288
  Lactobacillus, 260, 266–271, 273
  Lactococcus, 416, 424
  Listeria. *See* Pathogens
  Micrococcaceae, 256, 266, 272, 316
  Pediococcus, 260, 266, 268, 316
  Penicillium. *See* Mold
  Pseudomonas. *See* Pathogens
  Salmonellae. *See* Pathogens
  Staphylococcus, 266–267, 272, 288, 316
  Mycobacterium tuberculosis, 226
  Pseudomonas, 219
  Rhizoctonia, 218
  spoilage, 218, 220
  Staphylococcus aureus, 219
  thermal resistance, 218, 220
Microsatellites, 473
Microwave, 873, 876, 883
Milk, 472–473, 488–498, 501–502, 504, 509–510
  acidophilus, 456
  ash content, 349
  beverage, 474
  caprine, 463, 468, 469
  chemical composition of, 349
  composition, 472
  constituents, 349, 461
  defatted, 478
  definition, 349
  factors affecting composition, 349
  fat depression, 459
  minor constituents, 365
  physical structure, 349, 366
  price, 452
    feed price, 454
  production, 454, 456
  products, 451
  protein, 464
  raw, 457
  skimmed, 478
  supply, 450
Milk and milk products, flavors of, 716
Milk constituents, 349
  major constituents, 351
    biogenic activity, 105
    fat, 349
    lactose, 362
    minerals, 363
    proteins, 353
    water, 349
Milk enzymes, 360
  alkaline phosphatase, 361
  lipoprotein lipase, 361
  lysozyme, 361
  protease, 361
Milk fat, 106, 349, 351
  cholesterol, 351
  concentrated source, 597
  conjugated linoleic acids, 106
  constituents, 350
  fat globule membrane, 351–352
    composition, 352
  fatty acid profile, 351
  phospholipids, 106, 351, 355
Milk fat content, 337
  low fat products, 337
  skim milk products, 337
  thermalized cream, 337
  whole milk products, 337
Milk, mineral composition, 364
Milk physical properties, 364
Milk proteins, 105, 355
  caseins, 355–356
    alcohol coagulation, 364
    heat coagulation, 358
    isoelectric precipitation, 100, 357
    peptides, 101, 360
    physical and chemical properties, 359
    polyvalent ion precipitation, 358
    rennet coagulation, 358

functional attributes, 362
whey/serum proteins, 358–359
peptides, 101, 360
Milking
year-round, 461
Mill run, 1057
Mince, 877, 881, 885
Mineralizations, 473
Minerals, 363, 456, 466
calcium, 468
chloride, 468
chlorine, 468
magenesium, 468
Mo, 469
partition in milk, 365
phosphorus, 468
potassium, 468
selenium, 469
sodium, 468
sulfur, 468
zinc, 469
Misshapen, 1053
Mix calculations, 606
arithmetic method, 607
milk and cream formula, 610
serum point method,
Mix freezing, ice cream, 619
hardening, 624
overrun, 621
refrigeration, 620
types of ice cream freezers, 622
Mix processing, aging, 615
blending, 613
flavors, 617
homogenization, 616
pasteurization, 615
Mix proof sheet, 611
Modifications, 476
Modified atmosphere packaging, 261–262
Mohair, 454
Moiety, 480
Mold. 640–641, 644. *See also* Molds
Molds, 268–269, 273, 304, 477, 653, 658, 661, 664
Molybdenum, 458
Monoglycerides, 606
Monosaccharides, 468
Monosaturated, 385
Mozzarella, 509
Mucins, 157
Multiprenyl-menaquinones, 49, 50
Multitude, 476
Muscle, 858–859, 860, 864, 876–880
actin, 875
fibers, 858, 860, 883
proteins, 877–879, 883
Muscle proteins, 777
gelling polysaccharides, 779
solubilization, 778
Mutants, 470
Mutations, 471
Mya arenaria, 898
Myocomma, 858
Myofibrillar proteins, 236, 256, 269, 271, 283
Myofibrils, 878
Myoglobin, 244, 256, 262, 315, 858, 882
Myoseptum, 858
Myotomes, 858

2,6-Nonadienal, 940
(E)-2-Nonenal, 726
National Marine Fisheries Service (NMFS), 822
National Shellfish Sanitation Program (NSSP), 949
National Yogurt Association
live and active culture seal, 649, 668
Natural breeding, 461
Neutrophil, 389
Nitrate, 236, 252, 256, 262, 267–268, 282, 316
Nitrate reductase, 236, 267–268
Nitric oxide, 244, 315
Nitrite, 236, 242, 252, 256–257, 262, 267–268, 282, 304, 306, 312, 315–316
Nitrogen moieties, 480
Nitrogenous compounds, 905
Nitrosamines, 257, 273
Nitrosomyoglobin, 244, 262, 308
NMFS, 818
Nonfat dry milk, 474
Nonfat ice cream, 595
Nonprotein nitrogen, 459, 465
North American freshwater catfish and catfish products, 849–852
Norvegia, 499, 501
Novelties, 630
Novelty category, 635
bottom filled, 640
cartoon characters, 643
extruded, 636, 644
fudge bars, 636
ice cream sandwich, 636, 642

Novelty category (*Continued*)
ice pops, 636
pudding bars, 638
quiescently frozen, 636, 637, 640, 641
stirred, 640
NRC, 451
NSSP, 947
Nubbin, 1053
Nubian, 382
Nucleotides, 284, 287, 289, 471, 858, 905
Nutraceutical, 125
minerals, 126
magnesium, 126–127
selenium, 126
vitamin C, 127
vitamin E, 127
zinc, 127
Nutraceutical products, 447
Nutrient composition, 476
Nutrient destruction, 220
Nutrition, 450, 477
basic, 450
human, 479
Nutritional, 458
manipulation, 459
Nutritional benefits of fermented milk, 105
Nutritional education and labeling act, 593, 623
Nutritional properties, 43–45, 47, 49
Nutritive value of ice cream, 630

Oberhasli, 382
Odors, 868, 880, 882, 884, 887
Off-flavor. *See* Off-odor
Off-flavors, 367
Off-odor, 235, 237, 311, 315
Oil, 463
canola, 463
seed, 463
Okra, frozen, 990–991
styles, 991
cut, 991
whole, 991
Oligonucleotides, 472
Oligosaccharides, 468, 690
Olive oil
consumption, 56
extra virgin, 60–62
heating, 59
phytochemicals, 58
supplementation, 62
Onion rings, frozen, 991
types, 991
French fried, 991
raw, 991
Opioid peptides, 491–493, 495–496
Optical analysis, 465
Optical properties, 366
Organic bases, 932
Organic milk, 458
Orotic acid, 456
Osmolarity, 385
Osmotic pressure, 376
Osteoporosis, 207–210
Ovine, 470
Oxidation, 235, 240–243, 245, 256, 261–262, 267, 272–273, 289–290, 305, 388, 390, 858, 877, 879, 880, 882, 884, 887, 888
Oxidation-reduction potential, 370
Oxidized flavor, 468
Oxygen, 858, 879, 882, 886, 887
Oyster
Eastern oyster, 898
Olympia oyster, 898
Pacific oyster, 898
processing, 898
Oysters, 954–955
Oystrea lurida, 898

P-cresol, 725, 733
Package, 861–868, 870, 874, 879, 886–887
Packages for milk products, 338
multilaminated packaging, 338
paper-board container, 342
plastic packaging, 338
Packaging, 242–243, 261, 406, 408, 409, 475
metal packaging materials, 904
paper and card packaging materials, 904
plastic packaging materials, 903–904
Packaging materials, 861–862, 867, 869, 874, 886–887
Packaging, frozen desserts, 624
heat shock, 628
manufacturing procedure, 626
retail display equipment, 627
retail handling, 628
storage on retail premises, 627
transportation, 627
warehouse equipment, 627
Pandalus
borealis, 895
dispar, 895

goniurus, 895
platyceros, 895
Panulirus
argus, 897
cuanus, 897
interruptus, 897
Paprika, 1031
carotenoids, 1032
color, 1032
dehydration, 1035
emerging technologies, 1036
milling, 1036
processing, 1034, 1037
quality, 1040
storage, 1037, 1039
Paralithodes camschatica, 897
aztecus, 893
brasileinsis, 895
monodon, 895
penaeus, 895
setiferus, 895
Paralytic shellfish poison (PSP), 949
Parasite(s), 950–952
Parathyroid hormone (PTH), 209
Parity, 380, 459
Parmigiano Reggiano, 509
Particulate
dried, 479
Pasteurization, 388, 394, 404–405, 468
HTST, 470
LTLT, 470
Pasteurized, 457
Pasteurized milk ordinance, 649, 655, 659
Pasteurizing, 477
Pathogenic
bacteria, 458
microorganisms, 474
Pathogens, 86, 91, 94–96
clostridium botulinum, 91, 219, 257
clostridium perfringens, 769
control, 26, 256
Escherichia coli, 157, 769, 809, 869
Listeria monocytogenes, 91, 142, 504
opportunistic, 94, 96
Salmonelle, 509
Pathological, 457
Peanut butter, 404
Peas, frozen, 991
black-eye, 992
field, 992
Pecorino Romano, 505, 509
Pectin, 436
high-ester pectin, 436
low-ester pectin, 436
protein stabilizing mechanism, 436
Peppers, 1059
Peppers, frozen sweet, 992
styles, 992
diced, 992
halved, 992
sliced, 992
unit, 992
whole stemmed, 992
whole unstemmed, 992
types, 992
green, 992
mixed, 992
red, 992
Pepsin, 493–495, 499, 501, 504–509, 723
Peptidases, 239, 269–270, 283
Peptides, 239, 245, 269–270, 283–286, 288, 856
Peptides, bioactive, 105, 106
Peroxide, 469
Pesticides, 947, 950, 953, 958, 962, 963
pH, 218, 220, 239, 241–242, 257, 262, 315, 322, 386, 388, 455, 474, 1046–1047, 1049
alkaline, 465
Phenolics, 1112
Phenylketonuria, 582
Phosphatase, 387
Phosphate, 480
Phospholipases. *See* Lipases
Phospholipids. *See* Lipids
Phospholipids, 461
phosphatidyl choline, 461
phosphatidyl ethanolamine, 459
phosphatidyl inositol, 462
phosphatidyl serine, 462
Phosphorus (phosphate), 213
Phosphorus, 388, 394
Phosphorylation, 471
Physical characteristics of milk, 366
acidity and pH, 368
buffering capacity, 368
colligative properties, 376
boiling point, 377
freezing point, 376
osmotic pressure, 376
curd tension, 375
density and specific gravity, 373

Physical characteristics of milk (*Continued*)
electrochemical properties, 370
electrical conductivity, 370
oxidation reduction potential, 370
flavor, 367
foaming, 374
optical properties, 366
color, 366
refractivity, 366
surface activity, 374
thermal properties, 370
effects of heat, 371
heat capacity, 370
heat stability, 371
specific heat, 371
thermal conductivity, 371
thermal expansion, 370
viscosity, 374
Physical equilibrium, 350
Physical structure, 218, 348
Physico-chemical, 447, 457
properties, 447, 457, 468, 470
Physiological, 458
Pickle ingredient, 1048, 1054
Pickle sizes, 1049
Pickle(s), 1046–1047
cured pack, 1047–1048
dill, 1047
mustard, 1047
relish, 1048
sour, 1047
sweet, 1047
fresh pack, 1048
dietetic, 1049
dill, 1049
relish, 1049
sweet, 1049
refrigerated, 1049
styles, 1046
cut, 1047
relish, 1047
sliced crosswise, 1047
sliced lengthwise, 1047
whole, 1046
whole mixed, 1046
Pigment. *See* Myoglobin
Pigment, 875, 881–882
Plain condensed milk, 602
Plant oil, 974
Plants and vegetables, 56
Plasma,
antioxidant activity 59, 60
lycopene 58, 59, 69
Plasmin, 387
Plastic film, 869
Polar bear, 385
Pollock, 932, 935, 940
Polydextrose, 635, 637
Polymerase chain reaction (PCR), 471–472
Polymorphism, 458, 464, 470
protein, 470
Polysaccharide, 383
Polysorbates, 606, 642
Polyunsaturated, 384
Popularity, 478
Population, 449, 451
human, 451
Porphyromonas gingivalis, 500, 504, 506
Positional isomers, 463
cis-, 463
trans-, 463
Potassium, 184, 202, 210, 388
Potatoes, 1014–1018
conditioned, 1015
desugaring, 1018
starch content, 1015
sugar, 1018
varieties, 1015
Potatoes, frozen French fried, 992–993, 1014–1018
fry color, 1020
length, 1022
manufacture, 1016–1020
blanching, 1018
by-products, 1017
desugaring, 1018
frying, 1018
peeling, 1016
sizing, 1017
slicing, 1017
trimming, 1017
washing, 1016
styles, 993
crinkle cut, 993
dices, 993
rissolé, 993
slices, 993
straight cut, 993
strips, 993
types, 993
institutional, 993
retail, 993
Potatoes, frozen hash browns, 994
styles, 994
chopped, 994

diced, 994
shredded, 994
Pouchitis, 166
Poultry, 751–762
boneless, 786
carnosine, 788
color, 756, 759, 789
color defects, 756
consumption, 784
cuts composition, 784
fatty acids, 788
feeding, 790–791
tocopherol, 790
vitamin E, 791
flavor, 757–758, 760–762, 790–793
antioxidants, 791–792
cholesterol, 789–790
lipid oxidation, 789
Maillard reaction, 788–789
off-flavor, 787, 788
warmer-over flavor, 788
frozen storage, 792–793
genotype, 790
lipid composition, 788
conjugated linoleic acid, 791
PUFA, 791
lipid oxidation, 764–765
MDP, 785
marination, 751–769
marinade ingredients, 756–766
acidulants, 764
antimicrobials, 766
antioxidants, 764
binders, 763
flavor enhancers, 762
flavor trends, 762, 769
formulation, 757
phosphates, 759
salt, 758
species, 760
sweeteners, 763–764
marinating systems, 767
microbiology, 766, 769
raw materials, 753
market, 753–754
meat quality, 754
packaging, 768, 793
per capita consumption, 752
pH, 792
postmortem, 792
preservation, 786
processing, 787
products, 752
proteins, 753, 755–756
slaughtering, 792
spoilage, 768
storage life, 786
texture, 755
water holding capacity, 755, 758–759
Poultry meat demand, 775
Powder, 404–407
Powdered, 479
Prebiotics, 110
Precursors, 43, 47
Premature
infant feeding, 480
Premium ice cream, 644
Pressure reduced, 479
Prevention of rickets, 41, 47
Primer, 471
Primex, 932–933
Probiotic
Bacillus cereus, 68
bifidobacterium sp., 68, 70, 77
biomarkers, 68
enterococcus faecalis, 68
escherichia coli, 68
lactobacillus sp., 68, 70, 76–77
microbes, 68
products, 73–75
Saccharomyces boulardii, 68
Saccharomyces cerevisiae, 68
strains, 69
survival, 70, 72
technology, 71
viability, 73–74
Probiotics, 92–93, 96, 110, 154–158, 162–163, 274, 478, 646, 667
Probiotic cultured milk products, 446
Probiotic cultures, 412, 422
Bififobacteriul bifidum, 417
bifidobacterium species, 412, 423
Lactobacillus acidophilus, 412, 418, 423
Lactobacillus casei, 412
Lactobacillus delbreuckii subsp. bulgaricus, 417–418, 421
Lactobacillus gasseri, 412
Lactobacillus johnsonti, 412
Lactobacillus reuteri, 412
Lactobacillus rhamnosus, 412
S. thermophilus, 417–418
therapeutic minimum, 423

Processing, 472
  characteristics, 472
Processing fluid milk products, 334–345
Processing procedures, 599
  aseptic processing, 335, 339–341
  direct heating, 335
  filling operation, 338
  high-temperature-short-time (HTST) continuous-flow pasteurizer, 335
  high-temperature-short-time (HTST) pasteurized milk products, 338
  HTST pasteurization process, 338
  in-container sterilization, 338
  maintaining temperature of finished product below 4°C, 388
  milk fat content, 337
  minimal temperature-time relationship, 336
  packaging, 338
  plate heat exchanger, 335
  postpasteurized contamination, 337
    biofilm formation, 337
  processing environment, 335
  receipt of raw milk at processing plant, 334
  steam infusion, 335
  steam injection, 335
  the pasteurization process system, 335
    heat exchangers, 335
    holding tube, 335
    timing pump, 335
Production, 451, 454
  record, 452
Prolactin, 467
Proteases. *See* Proteinases
Proteases, 480, 540
  heat stability, 546
  types, 540
Protective cultures, 93
Protein, 381, 458, 464–465, 857–858, 878–879, 883, 888
  amino acid profile, 577
  casein, 558–587
  coagulable, 467
  digestibility, 577
  emulsions, 573
  foams, 573
  gelation, 572
  hydration, 575
  loci, 471
  major, 471
  minor, 467
  modifier, 465
  nitrogen conversion factor, 580
  polymorphism, 470, 472
  solubility, 570
  surface activity, 572
  whey, 465, 471
Protein hydrolysates, 538
  analysis, 540
  applications
    bioactive peptides source, 539, 547–552
    hypoallergenic infant formulas, 539, 547–552
    nutrition support, 547, 550
    nutritional/protein supplements, 547–548
  definition, 539
  parameters
    AN/TN ratio, 541, 543, 550
    degree of hydrolysis (DH), 541, 550
    molecular weight distribution, 542, 548
    peptide chain length (PCL), 542
  production, 544
    post-hydrolysis processes, 546–548
  properties, 546, 550
Proteinase K, 502
Proteinases, 269
Proteins in milk, 693
  analytical techniques, 698
  human vs bovine, 695
  plant proteins for infant formulas, 698
Proteolysis and related reactions, 722
Proteolysis, 239, 245, 262, 270–271, 283–285, 296, 322, 491, 501, 502
Proteose, 465
  activity, 467
  peptone, 465
Proximate, 458
  composition, 458
PSE, 235
PSP, 949
Putrescine. *See* Amines
Pyrones, 726

Quality evaluation, 1023–1027
  frozen French Fried Potatoes, 1023
    flavor, 1023
    fry color, 1021–1023
    uniformity, 1024

Quality requirements, 1056–1059
Quality, 881
Quantitative variations, 470

Rabbit, 381
Radiation, 868
Rancid, 389, 394
Rancidity, 241, 256, 323, 877, 879–880, 885–886
Randomized Controlled Trials (RCTs), 159, 178
Rat, 390
Raw fish, 965
  brining, 965
Raw milk, 89, 96
Raw milk quality, 332–334
  added water, 334
  antibiotic residues, 333–334
  bacterial limit, 333
  caustic cleaner, 333
  farm bulk tank operation, 334
  grade A standards (USHHS), 332
  mastitis, 333
  preliminary incubation count, 334
  sediment grade standard, 333
  somatic cell count, 333
  standard methods for the examination of dairy products, 331
  temperature standard, 333
RDA, 41–42
  vitamin A, 43–44
  vitamin D, 47
  vitamin E, 46
  vitamin K, 50
Recombination, 472
Redox potential, 219
Redox system, 49
Refractive index, 472
Refractivity, 367
Refrigeration, 861, 865, 871–872
Regulation of genes, 46–49
Reindeer, 380–381
Renal stones, 202, 210
Rennet, 394–395
Rennetability, 470
Renneting, 472
  property, 472
  time, 473
Repercussions, 472
Restrictive environment, 156
Retinoids, 43, 44, 45
Ribonuclease, 467
  pancreatic, 468
Rigor mortis, 877
Ripening, 476
Ripening (of meat products), 237, 252
Ripples/variegates, 618
RNA splicing, 470
Romano, 389, 394
Root beer, 404, 406
Roquefort, 389, 394
Rotation, 479
Roughage, 459
Rumen, 470
Ruminant, 383

Saanen, 382
Saccharomyces cerevisiae, 495, 497
Safety, 871, 872
Sales, 635
Salinometer, 965
Salmon, 858, 882, 885
Salmon control plan, 949
Salmonella, 509
Salt, 236–239, 242–243, 256, 282, 287, 306, 311, 316, 322, 867, 876, 885–887
Salt equilibria, 474
Salt stations, 1056
Salt stock tanks, 1056
Salting, 236–237, 316, 477
Salty yogurt (laben), 433
Sample unit size, 1054
Sanitation, 218, 949–950, 952–958, 1020
Sanitizer(s), 859, 955, 960
Sanitizing, 951–952, 954, 957, 959, 962, 965
Saponification value, 472
Satiety, 583
Saturated, 384
Sausages, 252–256, 282, 285, 288, 290–296, 304
  cooking, 261
  drying, 261
  emulsification, 260
  fermentation, 260
  processing, 257–262
  ripening, 252–254, 258, 261
  traditional, 252
  types of
    Bologna, 254, 256
    chorizo, 254–255, 267

Sausages (*Continued*)
cooked sausages, 254–256
dry fermented sausages, 254, 315–316
Frankfurter, 254, 256, 282, 291–292, 296, 308–312
French, 252, 255
fresh, 253
German, 255
hot-dog, 254, 256
Hungarian, 255
Italian, 255
Lebanon Bologna, 254
longaniza, 254
pepperoni, 254–255
poultry, 256
raw fermented, 253–254
salami, 252, 254–255, 316
salchichón, 252, 254–255
semidry sausages, 254
summer sausage, 254–255
Wiener, 254, 256
Savory flavors, 931
Scallops, 932, 958–960
Scandinavian sour milk, 432
Scandinavian sour milk products, 415–416
Seafood byproducts, 930–931
Seafood byproducts flavors, 932
amino acids, 935
cucumber and green-like odors, 940
enzymatic hydrolysis production, 933
malty, caramel, and sweet-like odors, 940
mushroom-like odor, 941
other odors, 947
peptides, 936
popcorn-like odor, 938
potato-like odor, 938
volatiles, 937
Seafood byproducts flavorants, 929–940
Seafoods, frozen, 818
fish fillet blocks, 826–828
fresh and shrimp, 840–843
fried fish portions, 839–840
fried fish sticks, 837–839
halibut steaks, 823–824
headless dressed whiting, 822–823
minced fish blocks, 829–832
North American freshwater catfish and catfish products, 849–852
raw breaded fish portions, 835–837
raw breaded fish sticks, 833–835
raw breaded scallops and fried scallops, 847–849
raw breaded shrimp, 843–846
raw fish portions, 832–833
raw scallops, 846–847
salmon steaks, 824–825
Season, 457, 459, 461
Seasonal, 457
deviation, 461
Seasonings, 257
Sediment, 455
Selenium, 456
Semidry-fermented sausages. *See* Fermented sausages
Sensory
descriptive terms, 306–323
quality, 240–241, 243–244, 262, 273, 302–322
Separators, 479
Sequenced, 463
Serum albumin, 463
bovine, 463
Serum protein, 463
Serum solids, 549
Shape, pickles, 1051
crooked, 1051
curved, 1051
defect, 1051
diameter, 1051
end cut, 1052
Sheath, 858
Sheep, 380, 385–387, 389, 392, 450, 454
Shelf life, 218, 407–409, 879, 880, 884–887
Shelf line, 470
Shelf stability, 881, 887
Shellfish freezing
by-products of frozen shellfish, 908–909
effects of freezing on physical properties, 904–907
freezing, 894
freezing methods, 899–902
glazing, 902
harvesting, 895–898
microbiological quality, 907–908
nutritional values, 896
packaging, 903
quality control and assurance, 909–912
thawing, 902–903
varieties, 896–898

Sherbet, 595, 641, 644
Short-chain fatty acids, 161
Shrimp(s), 882, 883, 960–964
  aquacultured shrimp, 895
  cold water shrimp, 895
  freezing requirement, 896
  melanosis, 905
  mushiness, 906
  saltwater shrimp, 895
  warm water shrimp, 895
Signaling, 158
Size distribution of milk fat globules, 353
Skyr, 420, 424–425
Smoke. *See* Smoking
Smoked fish, 964–967
Smoking, 237, 240–241, 252, 254–255, 257, 260–261, 282, 290, 296
Sodium, 161, 210, 1051
Sodium alginate, 404, 406
Sodium carboxymethyl cellulose, 605
Sodium caseinate, 494, 501, 509
Sodium chloride. *See* Salt
Solids, 457, 637, 639, 644
  total, 459
Solid-not-fat (NSF), 458, 459
Somatic cell counts, 457
Somatic cells, 389, 394, 395
Sorbet, 596, 641
Sorbitol, 637
Sotolon, 726, 727
Sour cream, 478, 521
  analysis, 532
  chemical and physical background, 520
  consumer safety, 532
  cultures, 524
  economical significance, 522
  history, 520
  ingredients and additives, 523
  legal definitions, 521
  manufacturing, 526
  packaging, 530
  quality, 532
  usage, 531
Sour dip, 478
Sources of vitamins, 41
  vitamin A, 43
  vitamin D, 47
  vitamin E, 46
  vitamin K, 50
Soy, 393
Soy proteins, 698
Soymilk, 1064, 1065
  grinding, 1067
  heating and separation, 1068
  preparation, 1065
  quality, 1081–1082
  solids content and ph, 1070
Specialty gourmet, 451
Species, 450, 456, 459
Specific gravity, 373, 1016
Specific heat, 371
Sphingomyelin, 461
Spices, 255, 257, 287, 290, 294–295, 311, 316
Spisulla solidissima, 898
Spoilage, 236, 243, 260, 857, 859, 877–882
Spontaneous lipolysis, 463
Sports nutrition, 582
Stability, 868–876, 881, 884, 886–887
Stabilizers, 404–407, 479, 596, 604, 637, 642
Stage of lactation, 459, 468
Standardization, 474, 477
Staphylococcus aureus, 333, 500, 504–505, 508–509
Staphylococcus carnosus, 500, 504–506
Static headspace-olfactometry, 732
Starch, 245, 262
Starter adjuncts, 92, 93
Starter cultures, 86–89, 92, 96, 256–258, 266–270, 285
Steam-heated, 479
Steatorrhea, 480
Sterilized, 478
Stick, 635–636
Stoke's law, 363
Stomach acid, 478
Storage, 218, 478, 869, 872
Str. creamosis, 437, 444
Str. lactis, 437, 444
Str. thermophilus, 440
Strained yogurt (labneh), 443
Strecker, 245, 270, 287–289, 296
Strecker reaction, 932
Strep. diacetylactis, 437, 444
Streptococcus mutans, 490, 504, 506
Streptococcus pyogenes, 504
Streptococcus thermophilus, 103, 412, 477, 649, 652, 659, 664, 666, 669, 652–653, 656, 661
Structure of vitamins
  vitamin A, 42–44

Structure of vitamins (*Continued*)
vitamin D, 47–48
vitamin E, 45, 47
vitamin K, 49–50
Stuffing, 258
Subsistence, 450
Substitution, 470–471
Substrate, 465
Sucrase-isomaltase, 168
Sucrose, 479, 603, 637–639, 644
Sugar alcohols, 602
Sugar syrup, 478
Sugar, 636
Supercooling, 860, 862
Supplementary
feeding, 454
Supplementation, 463
Surface activity, 375
Surface-ripened, 476
Surface tension, 478
Surimi-based products, 933
Sweet caramel, 478
Sweeteners, 402–404, 479, 602, 637
non-nutritive, 604
nutritive, 602
Swordfish, 882
Symbiotic
lactic acid bacteria, 478
Synbiotics, 71, 106
Syneresis, yogurt, 652–653, 656, 661
Synergy, 44–46
Syrup, 636–637, 639, 644

Taste compounds in milk and milk products, 736
Taste, 236, 239, 256
Taurine, 858
Temperate climate, 460
Temperature, 884, 885
Tenderness. *See* Texture
Texture, 237, 239, 243, 245, 262, 306–321, 823–824, 828–829, 831–832, 883, 1053
Texture and physical appearance, 924
Thaw, 873–874, 881
Therapeutic values, 479
Thermal conductivity, 371
Thermal expansion, 370
Thermal processing, 225, 227
aseptic processing, 229
canning, 229
cooking, 218, 225
D-value, 223
F-value, 224
pasteurization, 225
radiation, 222
scalding, 225
sterilization, 218, 225
z-value, 224
Thermally-induced changes, 717
Thermal properties, 370
Thermolysin, 494, 500, 502
Thiazole, 717, 939–941
Thin film, 479
Thiocyanate, 388
Threshold, 464
Thrombin, 494
Time-temperature, 955, 958, 960
Tissue, 857–858, 860, 881
Titratable acidity, 478
Titration, 465
curve, 465
TMA. *See* Trimethylamine, 942
TMAO. *See* Trimethyl amine oxide, 858
Tocopherol, 45–47, 51
Tocotrienol, 45
Tofu processing, 1071–1072
coagulation, 1070
coagulant concentration, 1072
coagulation method, 1073
coagulation temperature, 1072
soymilk concentration, 1070
type of coagulants, 1071
pilot-scale method, 1082
pressing, 1074
small-scale method, 1082
soaking, 1065
Tofu, 1065, 1066, 1067
cultivar/material effect, 1074
chemical composition, 1081
identity-preserved, 1074, 1081
size and hilum color, 1081
soybean quality, 1081–1082
isoflavones, 1079
lipoxygenases, 1080
mechanism of curd formation, 1080–1081
lipids, 1077
phospholipids, 1077
phytate, 1078
protein charges, 1081
quality, 1082
saponins, 1079
soy storage protein, 1076, 1077
beta-conglycinin (7s), 1077

gelation, 1078
glycinin (11s), 1077
ratio of 11s/7s, 1077
storage effect, 1075, 1076, 1082
type, 1071
Toggenburg, 382
Tomato, 1109
dehydration, 1110
dried, 1109, 1113
nutritional composition of, 1111–1112
solar drying of, 1115
spray drying of, 1117, 1118
sun drying of, 1115
Tomato catsup, 1105–1106
Tomatoes, 58–62
Tomato concentrate, 1101–1104
aseptic processing, 1102
break, 1101
color, 1103
pectinmethylesterase, 1101
polygalacturonase, 1101
quality standards, 1103
Tomato juice, 1104–1105
aseptic processing, 1105
break, 1104
Tomato processing, 1091–1107
biology, 1093
lycopene, 1092
Lycopersicon esculentum, 1093
nutritional value, 1095
Tonnage, 451
Total solids, 595
Total volatile basic nitrogen (TVB-N), 909
Toxicity, 41, 42, 44
Toxins, 880
Traditional lactic bacteria, 412
mesophilic lactic bacteria, 412
thermophilic lactic bacteria, 412
Transamination, 268, 270
Transcriptional regulators, 44
Transferrin, 467
Transformation, 479
Translocation, 157
Triacylglycerides, 382
Triacylglycerols. *See* Lipids
Triglycerides, 472, 480
Trimethylamine (TMA), 909
Trimethylamine oxide (TMAO), 858, 879, 883–886, 905
Tripeptidyl peptidase. *See* Peptidases
Trypsin, 491, 493–494, 496, 498–500, 502, 504, 507, 509
Tuna, 857, 882
Tunnel, 865, 867
Tyramine. *See* Amines
Tyrosine, 237, 239, 270, 273

U.S. Food and Drug Administration (FDA), 1046, 1049
UHT, 454, 470, 474
Ulcers, 480
Ultrafiltration (UF), 416
Underdeveloped, 450
Underfeeding, 454
Undernutrition, 451
Uniformity, 1053
Unit, 1053
United States Department of Agriculture, (USDA), 986, 1054
standards for grades of pickles, 1046
Unmolding, 477
Unsaponifiable, 463
matter, 472
Upper limit of safety (UL), 202
Uric acid, 467
USDA Agricultural Handbook, 476
USDA colors, 1024
USDA, 950, 1016, 1019

Vacuum, 479
Vacuum-packaging, 261–262
Vancomycin-resistant enterococci, 94
Vanilla, 404, 406, 409, 618, 619
Variants, 470
Vegetable, 56, 59, 62, 406
Vegetables, mixed frozen, 994–995
kinds, 994
proportions, 995
Vegetarians, 202, 212
Venus mercenaria, 898
Veterinary, 456
Viili, 412, 424–425
Virulence factors, 93–95
Viscosity, 374, 477, 637, 639, 641
Vitamin D, 202–204, 205
from foods, both natural and fortified, 209, 212
from supplements, 202–204
skin biosynthesis, 209, 211–212
Vitamin D metabolites, 205
25-hydroxyvitamin D, 209
calcitriol (1,25-dihydroxyvitamin D), 205

Vitamins, 365, 371, 388, 394, 458, 467
A, 469
B12, 470
C, 469
D, 469
folic acid, 470
niacin, 469
pantothenate, 469
pyridoxine (B6), 470
riboflavin, 469
thiamin, 469
VLDL, 383
Volatile compounds. *See* Flavor

Water, 381, 457, 874
activity, 219, 254
diffusion, 237–238
holding capacity, 235, 245
ice, 595
loss, 235, 243, 252
Weight control, 180, 203, 207
Weight loss, 184, 185, 207
Weight reduction, 106
Whale, 381
Whey, 381, 467
Whey protein, 465, 650, 652, 657, 660, 662
denaturation, 653, 696, 698
Women's Health Initiative (WHI), 204
Wrappers, 869

Xanthine oxidase, 468
bovine, 468
caprine, 468
Xylitol, 639

Yeast, 154, 267–270, 272–273, 287, 652, 656–659, 662
Saccharomyces boulardii, 154
Yersinia enterocolitica, 509
Yield, 452, 456
Ymer, 416, 432
Yogurt, 391, 412, 417, 432, 450, 474, 477, 495
bacteriophage, 653, 659
bio-yogurt, 412, 422
concentrated/strained yogurt, 432
classification, 412
Bulgarian buttermilk, 418
concentrated/strained yogurt, 420–421
drinking yogurt, 420
set yogurt, 419
stirred yogurt, 419
consumption, U.S. per capita, 648
culture, 653
characteristics, 667
Lactobacillus bulgaricus, 649, 652, 658, 659, 665, 667
live and active seal, 649, 668
probiotic, 648, 669
rods to cocci ratio, 652, 666
ropy, 655, 660, 666
Streptococcus thermophilus, 649, 652, 658, 659, 665, 667
survival, 667
cup set, 648, 650, 665
definition of, 648
drinks, 649, 661
drinking yogurt, 432
effect of strength and weakness of milk components used in production, 435
fermentation, 654–655, 664
cup set, 648, 650, 656
endpoint, 656, 660
post-fermentation acidification, 664
slow, 652–653, 659
stirred style, 650, 656
temperature, 655–656, 660, 665
frozen, 600
frozen yogurt processing, 443
gel strength
ingredient effect, 652, 664
processing effect, 655, 660, 662
ingredients
fruit preparation, 654, 658
incorporation of, 654
milk, 649
stabilizer, 653, 661
sweetener, 653–654
long-life drinking processing, 442–443
natural yogurt, 436
nutritive value, 666
organoleptic defects, 657–658
packaging and storage, 659
petit suisse/fromages frais, 434
probiotic yogurt, 412, 434
processing
heat treatment, 652, 660, 662
homogenization, 654
shear, 660
quality control, 659
set-yogurt, 432

set yogurt processing, 441–442
shelf life, 649, 658, 664, 668
stirred yogurt processing, 441–442
sweetened and fruit flavored yogurt, 412
texture
casein micelle, 662, 663
grainy, 654
ropy, 655, 660, 664
weak gel, 655, 660
yogurt mousse (aerated yogurt), 434

(Z)-1,5-octadiene-3-one, 732
(Z)-2-nonenal, 732
(Z)-4-heptenal, 2-acetyl-1-pyrroline, 728
Zinc, 473, 993, 1009

Cumulative Index

Volume numbers appearing in italics refer to volume subject material as stated in footnote.

2-Acetylthiazole, *1*: 458
2-Alkanols, *1*: 186
2-Alkanone, *1*: 186
2-Acetyl-1-pyrroline, *2*: 294, 727–728, 732, 938–939
2-Acetyl-2-thiazoline, *2*: 295, 728, 731
α-Acetohydroxy acids, *1*: 477–478, 488
α-Acetohydroxybutyrate, *1*: 488
α-Acetolactate, *1*: 477, 479, 483, 485, 488, 490
α-Acetolactate decarboxylase, *1*: 478
α-Amino N, *2*: 467
α-Amylase, *1*: 449–451, 457
$1\alpha,25(OH)_2D_3$, *2*: 47–49
β-Amylase, *1*: 447, 449–451
AAD gene, *1*: 473
Absorption, *2*: 480
  nutrient, *2*: 481
ACE-inhibiting, *2*: 110
Acesulfame K, *1*: 310
Acetaldehyde, *1*: 182, 186, 471, 479, 481, 851–859, *2*: 104, 660, 667
Acetate, *1*: 475
Acetic acid, *1*: 729–730, 735
Acetoin, *1*: 471, 478, 717, *2*: 271
Acetone, *1*: 212
Acid, *2*: 1050
  low-acid canned foods (LACF), *2*: 948
  low acid foods, *2*: 954
Acid (also acidity, also titratable acidity), *1*: 568–569, 572–574, 576, 578–579
Acid degree value (ADV), *2*: 463
Acid food(s), *1*: 225, 235
Acid index, *2*: 235–256
Acid-coagulated cheeses, *1*: 636
Acidification, *2*: 477. *See also* Fermentation
Acidified, *2*: 480
Acidity, *2*: 478
Acidity and pH, *2*: 368
Acidophilus, *2*: 391, 478
Acidophilus milk, *1*: 62–63, *2*: 412
Aconitase, *1*: 476
Actin
  aggregation complex of, *2*: 878
  carp, *2*: 858, 882

Handbook of Food Products Manufacturing: Principles, Bakery, Beverages, Cereals, Cheese, Confectionary, Fats, Fruits, and Functional Foods (Vol. 1)
Handbook of Food Products Manufacturing: Health, Meat, Milk, Poultry, Seafood, and Vegetables (Vol. 2)

Handbook of Food Products Manufacturing. Edited by Y. H. Hui

Actin (*Continued*)
denaturation, *2*: 879
interacting with metal ions, *2*: 879
muscle, *2*: 877–878
thin filament, *2*: 878
Active amyl alcohol, *1*: 471, 473
Actomyosin
amount of extractable, *2*: 878
ATPase activity of, *2*: 878
changes in muscle, *2*: 876
dissociation of, *2*: 878
during frozen storage, *2*: 878
extractable, *2*: 878
soluble, *2*: 878
viscosity of, *2*: 878
Acyl-coenzyme A, *1*: 474
Additives, *1*: 609, *2*: 881, 885
Adenosine dinucleotide phosphate, *2*: 381
Adenylylsulphate kinase, *1*: 481
Adjunct culture, *1*: 572–573, 578
Adjuncts, *1*: 447, 457, 468, 478
Advantages, *2*: 479
AEDA. *See* Aroma extract dilution analysis
Aeration, *1*: 588
Aflatoxin contamination, *1*: 698
Aftertaste, *1*: 288, 293
Agaricus bisporus, *1*: 183
Agglutinin, *2*: 385
Aggregation, *1*: 578, 653, 658
breakdown of TMAO, *2*: 879
electron microscopic observation, *2*: 878
formation of, *2*: 878
muscle protein, *2*: 878
myofibrillar protein, *2*: 878
Aging, *1*: 584
Aging time, *2*: 476
Agitation, *2*: 477
Agricultural business, *2*: 450
AGT1 gene, *1*: 470
Alanine, *2*: 15, 269–270, 285–286, 289, 465, 508, 579, 905, 936
Alcohol acyltransferase, *1*: 474
Alcohol dehydrogenase, *1*: 472–473, 478
Alcoholic beverages, *1*: 181–182
Alcoholic fermentation, *1*: 181
Alcohols, *1*: 181, 186, 210, 849, *2*: 478
Aldehydes, *1*: 181, 186, 210
Alitame, *1*: 310
Alkaline phosphatase, *2*: 361, 466–468
Alkalinity, *2*: 479
Alkyl-pyrazines, *2*: 938
Alleles, *2*: 470–471
specific primers, *2*: 471
Allelic
genotype, *2*: 471
variants, *2*: 470
Allergen(s), *1*: 284, 299
Allergic, *2*: 393
Allergies, *2*: 166, 450, 454, 479
Allergy, *2*: 118
classification, *2*: 118
definition, *2*: 118–119
mechanism, *2*: 115, 119–120
prevention, *2*: 123
therapy, *2*: 126
Alliin, *1*: 916
Allyl isothiocyanate, *1*: 125
Alpha lactalbumin, *2*: 386. *See also* Whey protein
Alpha-linolenic acd (ALA), *1*: 915
Alpine, *2*: 382
Alternative (new) technologies, *1*: 38–42
Alternative sweeteners, *1*: 310
acesulfame K, *1*: 310
alitame, *1*: 310
aspartame, *1*: 310
neotame, *1*: 310
sucralose, *1*: 310
Alzheimer's disease (AD), *1*: 914, 920, *2*: 497
Amaranth, *1*: 1023–1024
American Bottlers of Carbonated Beverages (ABCB), *1*: 421
American Heart Association, *1*: 919
American Spice Trade Association, *1*: 214
American whiskey, *1*: 517
Bourbon, *1*: 517
charring, *1*: 518
corn whiskey, *1*: 518
distillation, *1*: 518
column still (patent still), *1*: 518
Kentucky bourbon, *1*: 518
maple charcoal, *1*: 518
neutral grain spirit, *1*: 518
rye whiskey, *1*: 518
sour mash, *1*: 517
backset (setback), *1*: 517

sweet mash, *1*: 518
Tennessee whiskey, *1*: 518
American-type Camembert cheese manufacturing, *1*: 56, 58
AMF mixer, *1*: 321
Amines, *2*: 257, 270, 273, 288
Amino acid, *2*: 387, 465, 471
alanine, *2*: 465
anabolic pathway, *1*: 472–473
aspartate, *2*: 465
brach-chain (BCAA), *2*: 465
catabolic (Ehrlich) pathway, *1*: 472–473
essential (EAA), *2*: 465
glutamate, *2*: 465
glycine, *2*: 468
isoleucine, *1*: 472–474, 477
leucine, *1*: 472–474, 477, *2*: 465
lysine, *2*: 465
methionine, *1*: 478, 480–481
phenylalanine, *1*: 473
proline, *2*: 465, 468
serine, *2*: 468
sulfur, *2*: 465
threonine, *1*: 478, 484
total, *2*: 465
tryptophan, *1*: 473
tyrosine, *2*: 465
valine, *1*: 472–474, 477, *2*: 465
Amino acids, *2*: 239, 245–246, 262, 268, 272, 283–289, 316, 322
generation of. *See* Proteolysis
Amino acids metabolism, *1*: 472, 475, 477
Aminopeptidase N, *2*: 723, 725
Aminopeptidases, *2*: 239, 245, 270, 272, 287
Aminotransferase, *1*: 1027
Ammonia, *2*: 268, 287
Ammonium, *2*: 731, 737, 905
Amount of alcohol in candy, *1*: 699
Amplified, *2*: 472
Amplify, *2*: 471
Amyl alcohol, *1*: 182
Amylases, *1*: 188
Analytical requirements, *2*: 1055
Anaphylaxis, *2*: 118
Anatomical, *2*: 458
Anemic, *2*: 481
Anethole, *1*: 210
Angel food cake, *1*: 317
Angiotensin-I converting enzyme (ACE), *2*: 497–498, 501–503, 510
Angora, *2*: 454
Animal and marine fats and oils, *1*: 107–108
Anise, *1*: 210, 213
Annatto. *See* Color
Antagonism, *2*: 452
Antagonistic, *2*: 478
Anthriscus sylvestris, *1*: 167
Antiaging mechanisms, *2*: 136, 138
anticancer activities, *2*: 141
antioxidant activities, *2*: 136
cardiovascular protection, *2*: 140
eye protection against cataract and macular degeneration (MD), *2*: 138
metal-chelating activities, *2*: 138
neuroprotection, *2*: 138
Antibiotic resistance, *2*: 93–95
Antibiotics, *2*: 166, 457, 650, 660, 665, 667
Anticarcinogenic, *2*: 163
Antidiabetic, *2*: 176, 179, 181, 185
Antihypertensive peptides, *2*: 497
Anti-inflammatory properties, *2*: 47
Antimicrobial agents, *2*: 885
Antimicrobial compounds. *See* Bacteriocins
Antimicrobial metabolites, *2*: 156
Antimicrobial peptides, *2*: 503, 505, 509–510
Antimicrobials, *1*: 885–886, *2*: 220
carbonyl compounds, *1*: 885
essential oils, *1*: 885, 886
organic acids, *1*: 885
Antimycotic agents, *1*: 573
Antiosteoporotic and antiobesity effects, *2*: 107
Antioxidants, *1*: 129, 209, 993, 998, 1005, *2*: 44–47, 1111
activity, *2*: 60
application of, *2*: 1114
Chinese medicine, *2*: 56
functional food, *2*: 61
intake, *2*: 57
isoflavones, *2*: 58
olive oil, *2*: 62
plasma, *2*: 62
synthetic, *2*: 57
tomato, *2*: 59, 62
Antithrombotic peptides, *2*: 496
Apple cider, *1*: 856

Apple juice, *1*: 848–860
Apple: production, chemistry and processing, *1*: 773–791
  apple varieties, *1*: 777
  composition, *1*: 780
  consumption, *1*: 775
  harvest and storage, *1*: 776
  leading country, *1*: 774
  leading fruits, *1*: 774
  organic apples, *1*: 774
  physico-chemical characteristics, *1*: 779–783
    apple flavor, *1*: 779
    nutritional quality, *1*: 782
    phenolic compounds and antioxidant capacity, *1*: 780
  post harvest handling, *1*: 776–777
  processed apple products, *1*: 783–789
    apple juice and cider, *1*: 784–786
    apple slices, *1*: 786
    applesauce, *1*: 787
    dried apples, *1*: 787
    freeze-dried, *1*: 789
    glass transition, *1*: 787
    infused dried, *1*: 789
    processed frozen apples, *1*: 787
    water activity, *1*: 789
  supply, *1*: 774
Apples, *1*: 841–854
Aquaculture, *2*: 969–979
  cage-culture, *2*: 972–973, 975
  extensive, *2*: 970
  feeds, *2*: 971, 973–974, 976–977
  flesh quality, *2*: 975–976, 978
  intensive, *2*: 971–979
  polyculture, *2*: 969
  pond-culture, *2*: 969, 977–978
  production, *2*: 970–971
  selective breeding, *2*: 975
  semi-intensive, *2*: 970
Arginine, *1*: 977, 1004
Aroma, *1*: 279, 293. *See also* Flavor
Aroma extract concentration analysis, *2*: 731
Aroma extract dilution analysis, *2*: 726–727, 731
Aromatic herbs, *1*: 209
Aromatic profiles, *1*: 141
Artisanal, *2*: 454
Ascomycetes, *1*: 183
Aseptic packaging, *1*: 858–859
Aseptic processing of milk, *2*: 333, 339–342
  concept, *2*: 339
  equipment design and operation, *2*: 339
  extended-shelf-life milk, *2*: 341
  gelation phenomenon, *2*: 342
  milk system stability considerations, *2*: 342
  monitoring of thermal process, *2*: 341
  packaging platforms, *2*: 342
  product shelf life, *2*: 342–343
  the system, *2*: 339–341
  ultra-high-temperature (UHT) processing, *2*: 341
Ash, *2*: 381
Ash level, *1*: 216
Asian (Oriental) noodles
  classification, *1*: 541
  history, *1*: 539–562
  variables affecting quality, *1*: 561–562
Asparagus, frozen, *2*: 986
  styles, *2*: 987
    cut spears or cuts and tips, *2*: 987
    spears or stalks, *2*: 987
  types, *2*: 987
    green, *2*: 990
    green-white, *2*: 987
Aspartame, *1*: 310
Aspartic, *2*: 285, 322, 468, 508, 579, 935–936
Aspergillus, *1*: 188
Aspergillus niger, *1*: 186–188, 884
Aspergillus parasiticus, *1*: 187
ASTA color value, *1*: 217. *See also* American Spice Trade Association
ATF1 gene, *1*: 474–475
ATF2 gene, *1*: 474–475
Atherosclerosis, *2*: 468
Atomization, *2*: 479
ATP. *See* Nucleotides
ATPase
  activity, *2*: 870
Aureobasidium, *1*: 186
Authenticity, *1*: 215
Autoclave, *2*: 478
Autoxidation, *2*: 880, 886
Available water, *1*: 216
Ayrshire, *2*: 381
Azeotropic distillation, *1*: 159

2,3-Butandiol, *2*: 719
2,3-Butanedione, *1*: 477, *2*: 719, 727–732
Bacillus megaterium, *2*: 509
Bacillus subtilis, *1*: 187
Bacteria, *2*: 154, 457, 949, 953, 956, 958
  Clostridium difficile, (*C. difficile*), *2*: 163, 166–167
  coliform, *2*: 457
  count, *2*: 457
  E. coli, *2*: 154
  H. pylori, *2*: 156
  L. acidophulus, *2*: 156
  lactic acid, *2*: 154, 168
    Bifidobacteria, *2*: 154, 163, 166
    Lactobacilli, *2*: 154, 166
    Streptococci, *2*: 154
  listeria, *2*: 457
Bacteriocin, *2*: 268
Bacteriophage, *1*: 487, 572, 574, 588, *2*: 653, 659
Bacteriostatic, *1*: 602–603
Bacterium lacticus longi (*Lactococus lactis biovar longi*), *2*: 416
Bactiocins, *2*: 156
  heat-laible, *2*: 156
  lantibiotics, *2*: 156
  nonlantibiotics, *2*: 156
Bags, *2*: 869
Bagel(s), *1*: 293–299
  evaluation, *1*: 299
  New York style, *1*: 295
  troubleshooting, *1*: 299
Baker Perkins homogenizer, *1*: 321
Baker's percent, *1*: 285–286, 294, 300
Bakery, *1*: 262–266, 271–274
  biscuit, *1*: 262, 264, 266–267, 269, 271–273
    checking, *1*: 264
    cracking, *1*: 264
  bread, *1*: 262–263, 265, 268–269, 271–274
    sourdough, *1*: 263
    whole grain, *1*: 262
    whole wheat, *1*: 262
  cake, *1*: 263–264, 271–272
    batter, *1*: 263
    emulsion, *1*: 263–264
  pastry, *1*: 265
Bakery products, *1*: 102–103, 183
Baking, *1*: 291, 297, 315–316, 321
Baking powder, *1*: 285, 289, 312
  double acting, *1*: 289, 312
  single acting, *1*: 312
Baking soda, *1*: 285, 312
Balancing ingredients, *2*: 602
Banana, *1*: 817–826, 867
  classification, *1*: 817
    Cavendish, *1*: 817
    Grand Nain, *1*: 817
    Gros Michel, *1*: 817
    Lacatan, *1*: 817
    Lady Finger, *1*: 817
    Musa acuminata, *1*: 817
    Señorita, *1*: 817
  cultivation, *1*: 817
    finger, *1*: 817–818
    hand, *1*: 817–818
    inflorescence, *1*: 817–818
    suckers, *1*: 817–818
    tissue culture, *1*: 817
  figs, *1*: 868, 872–873
  fingers, *1*: 819, 872
  flour, *1*: 868, 873
  harvest, *1*: 819–820
    inflorescence, *1*: 819
    latex, *1*: 819
    tramline, *1*: 819
  nutritional quality, *1*: 820
    potassium, *1*: 820
    starch, *1*: 820
    sugars, *1*: 820
  nutritional value, *1*: 837, 873
  phenolics and antioxidant capacity, *1*: 821
    dopamine, *1*: 820
  post-harvest handling, *1*: 819–820
    chilling injury, *1*: 820
    color index scale, *1*: 820
    ethylene ripening, *1*: 819
    ripening, *1*: 820
  powder, *1*: 873
  production, *1*: 818–819
    yield, *1*: 819
  products and processing, *1*: 821–826
    chips, *1*: 821, 825
    individually quick frozen, *1*: 825–826

Banana (*Continued*)
osmotic dehydration, *1*: 825, 873, 875–876
other banana products, *1*: 821–826
puree, *1*: 821–825
storage, *1*: 820
supply, *1*: 818
Band ovens, *1*: 323
Basidiomycetes, *1*: 183
Batter(s), *1*: 225, 234, 291, 319–321
depositing, *1*: 291, 321
Batters and breading materials, *2*: 920
Beads
calcium alginate, *1*: 487
calcium pectate, *1*: 487
ceramic, *1*: 483, 487, 489
DEAE-cellulose, *1*: 487–490
porous glass, *1*: 487–488
Beef, *1*: 921
Beef extract replacer, *1*: 133
Beer, *1*: 174, 182, 195
alcohol-free, *1*: 487–489
bitterness, *1*: 456–457, 479
body, *1*: 451, 479
brewing process, *1*: 404, 444–446, 455, 485, 487
color, *1*: 450, 457–458, 466, 479
filtration, *1*: 447–449, 451, 455, 467
green, *1*: 446, 478, 481, 485, 488, 490
history, *1*: 444
low-alcohol, *1*: 488
packaging, *1*: 446
stabilization, *1*: 446, 483
Beet sugar, *1*: 104
Beiju, *1*: 186
Bell pepper, *1*: 219
Beneficial cultures, *2*: 102, 348
Beneficial microflora, *1*: 985
Benzaldehyde, *1*: 202
Benzoic acid, *1*: 917
Benzyl alcohol, *1*: 852
Beta-galactosidase, *2*: 391
Beta-hydroxybutyrate, *2*: 383
Beta-lactoglobulin, *2*: 386
Beta-lactoglobuline. *See* whey protein
Beverage technologists, *1*: 429
Beverages, *1*: 108–111
Bifidobacteria, *1*: 181, *2*: 101, 446–448, 490, 502
compounds, *1*: 998, 1000, 1002, 1004, 1006–1010
peptides, *1*: 991, 1007
Bioactive peptides, *2*: 6, 583
biological effects, *2*: 25
eggs, *2*: 21
fish, *2*: 22
functionality, *2*: 46
future applications, *2*: 28
meat, *2*: 22
milk and dairy products, *2*: 18
plants, *2*: 24
Bioactive peptides production
enrichment of bioactive peptides, *2*: 17
enzymatic hydrolysis, *2*: 9
microbial fermentation, *2*: 12
Bioavailability, *1*: 993–994, 1008–1010, *2*: 44, 47–48, 481
carotenoids, *2*: 60–62
Biochemical aspects, *2*: 857
Biogenic amines, *1*: 1007–1008. *See also* Amines
Biomedical, *2*: 454
Bioprocess, *1*: 170–171
Bioreactors, *1*: 198–200
packed-bed, *1*: 485, 487–488
Biosynthesis, *1*: 180
Bioterrorism Act of 2002, *1*: 246
Biotransformation, *1*: 180
Bis-(2-methyl-3-furyl)-disulfide, *1*: 859
Bitter, *1*: 611, 628
Bjerkandera adusta, *1*: 202
Black pepper, *1*: 212
Blanching, *1*: 225, *2*: 405, 1110
Blemished, *2*: 1051
Blend, *1*: 597, 605–606
Blood pressure, *2*: 210
Blue, *2*: 499, 501–502
Blue cheese, *1*: 600, *2*: 389
Blue cheese manufacturing, *1*: 55
Blue crab, *2*: 955
cooking, *2*: 955–956
cooling, *2*: 956
packing, *2*: 956
pasteurization, *2*: 956
pickling, *2*: 956

Blue whiting, *2*: 932
Blueberries, *1*: 805–812
  average production, *1*: 805
  blueberry products, *1*: 811
    blueberry juice and concentrate, *1*: 811
    blueberry puree, *1*: 811
    infused and stabilized blueberries, *1*: 811
    infused-dried, *1*: 811
    IQF blueberries, *1*: 811
  characteristics of selected varieties, *1*: 781
  demand, *1*: 807
  physico-chemical, phytonutrients, and nutritional qualities, *1*: 796, 808
    anthocyanins, phenolics and antioxidant capacity, *1*: 808
    chemopreventive and anti-adhesion properties, *1*: 810
    effect of processing on phytonutrient quality, *1*: 809
    flavor compounds, *1*: 808
    nutrients in blueberries, *1*: 810
Blueing, *2*: 905–906
Boiling, *1*: 297
Boiling point, *2*: 377
Bone, *2*: 44, 48–51, 208
  hip (proximal femur), *2*: 208
  lumbar vertebrae, *2*: 208
  nonvertebral sites, *2*: 208
Bone cells
  osteoblasts, *2*: 209
  osteoclasts, *2*: 209
Bone consolidation, *2*: 208
Bone formation, *2*: 208
Bone health, *1*: 923
Bone mineral content (BMC or bone mass), *2*: 208
Bone mineral density (BMD), *2*: 208
Bone resorption, *2*: 209
Bottled and canned soft drinks and carbonated waters, *1*: 110
Bound moisture, *1*: 163
Bovine, *2*: 386
  homolog, *2*: 465
  sequence, *2*: 471
Bovine serum albumin, *2*: 358
Bovine spongiform encephalopathy, *1*: 133
Bradykinin, *2*: 497, 501–502
Bread, *1*: 12, 66–70, 354
  Altamura bread, *pane di Altamura*, *1*: 367
  basic steps, *1*: 68
  bread substitutes, *grissini* and *taralli*, *1*: 368
  characteristics, *1*: 355
  functional ingredients, *1*: 67
  process, *1*: 359
  raw materials, *1*: 354, 356
  recipes, *1*: 69
  shelf life, *1*: 356, 358
  types, *1*: 66
  various breadmaking processes, *1*: 68
Bread and other bakery products except cookies and crackers, *1*: 102–103
Bread-making, *1*: 359
  sourdough, *1*: 361
  sponge and dough method, *1*: 361
  straight dough method, *1*: 360, 363
Breast milk, *2*: 120
  antibody, *2*: 120
  cytokine, *2*: 120
Breeding
  artificial, *2*: 461
  natural, *2*: 461
Breeds, *2*: 381, 449, 452, 459, 460
Brevibacterium linens, *1*: 181
Brie, *1*: 181
Brine, *2*: 241, 282
Brine cured ham manufacturing, *1*: 65
Brix, *2*: 1050, 1055
Brix/acid ratio, *1*: 852
Broccoli, frozen, *2*: 988
  styles, *2*: 988
    chopped spears or short spears, *2*: 988
    cut spears or short spears, *2*: 988
    pieces or random cut pieces, *2*: 988
    short spears or florets, *2*: 988
    spears or stalks, *2*: 988
Brown sugar, *1*: 310
Brown Swiss, *2*: 381
Browning, *1*: 9, 26, 27
  ascorbic acid, *1*: 9, 33
  citric acid, *1*: 34
  cysteine, *1*: 886–887
  enzymatic browning, *1*: 786, 821, 825, 887, 889
  phenolic substrates, *1*: 883
  polyphenol oxidase, *1*: 821, 886

Browning (*Continued*)
quinones, *1*: 883, 886
sodium erythorbate, *1*: 887
Browning Maillard, *1*: 301, 310, 318
Brussel sprouts, frozen, *2*: 986
Buck, *2*: 456
Buckwheat, *1*: 376–377
composition, *1*: 379
gluten-free bread, *1*: 381
pizzoccheri, *1*: 378
typical products, *1*: 369
Buffalo, *2*: 380–381, 384–385, 391, 451–452
Buffering, *1*: 602–603
Buffering capacity, *2*: 369, 479–480
Buildings, *2*: 859
Bulk density, *1*: 216
Bulking agents, *1*: 311
Bun tau, *1*: 559
Butanoic acid, *1*: 981
Butanol, *1*: 185
Butter, *1*: 705, 717–718, 721–722, *2*: 474
dried, *2*: 478
Butter clarified, *1*: 318
Butter drawn, *1*: 318
Butterfat, *1*: 653–655
clarified, *2*: 478
Buttermilk, *1*: 728, *2*: 391, 412, 415, 446, 474, 476, 606
cultured buttermilk, *2*: 415
cultured (fermented), *2*: 432, 444
processing, *2*: 443
traditional natural buttermilk, *2*: 415
Butyl acetate, *1*: 853–854
Butyric acetate, *1*: 184
Butyric acid, *1*: 182, 732, *2*: 383
Bypas fat, *2*: 383
By-product, *2*: 478

α_{s1}-Casein, *1*: 975
α_{s1}-CN, *2*: 491, 493, 495
α_{s2}-Casein, *1*: 973, 975
β-Carotene, *2*: 42, 44, 46, 469
β-Caryophyllene, *1*: 210, 853
β-Casein, *1*: 741, 973
β-CN, *2*: 491, 493–494
κ-Casein, *1*: 973–974
Caciocavallo, *2*: 509
Cadaverine. *See* Amines
Cadin-4-ene-1-ol, *1*: 187
Cajeta, *2*: 478
Cajun dishes, *1*: 211
Cake flour, *1*: 308
Cake mixing, *1*: 320
Cakes, *1*: 308, 314, 317
foam-style, *1*: 312, 317
shortened-style, *1*: 314, 316
Calcifications, soft-tissue, *2*: 202, 210
Calcified (mineralized) plaque, arteries, *2*: 210
Calcitriol (vitamin D metabolite; hormone), *2*: 205
Calcium, *1*: 602–603, 607–608, 619, 652, 654, 729, 743, *2*: 386, 388, 394
calcium chloride, *1*: 570–571, 576
calcium lactate crystals, *1*: 589
calcium phosphate, *1*: 570, 578, 581, 586, 589
from foods, both natural and fortified, *2*: 202
from supplements, *2*: 202
sequestrant, *2*: 474
Calcium, blood or serum, *2*: 209
parathyroid hormone, *2*: 209
regulation, *2*: 209
Calcium, functional role, *2*: 105–107
Calcium-sensing receptor (CaSR), *2*: 205
Callinectes sapidus, *2*: 897
Calories, *1*: 991–992, 1001
Calpains. *See* Proteinases
Calpis, *2*: 497, 501, 503
CAM, *2*: 177–178
Camel, *2*: 385
Camembert, *1*: 56, 181, *2*: 502
Canadian whisky, *1*: 518
base whisky, *1*: 518
flavoring whisky, *1*: 518
rye whisky, *1*: 518
Cancer, *1*: 913, *2*: 469
breast, *2*: 206
colon and rectal, *2*: 203
ovarian, *2*: 206
prostate, *2*: 205
Cancer magister, *2*: 897
Candida, *1*: 181, 183, 187
Candida cylindracea, *1*: 186
Candida utilis, *1*: 183, 186
Candy and other confectionery products, *1*: 104–105
deceptive products, *1*: 699

equipment and manufacturing processes, *1*: 698
raw materials, *1*: 696
sample collection, *1*: 699
Cane sugar except refining, *1*: 103–104
Cane sugar refining, *1*: 104
Canestrato Pugliese, *2*: 505–506, 509
Canned and cured fish and seafood, *1*: 111
Canned fruits, vegetables, preserves, jams, and jellies, *1*: 95–96
Canned tomatoes, *2*: 1096–1099
additives, *2*: 1098
blanching, *2*: 1098
firming agents, *2*: 1099
pectinmethylesterase, *2*: 1098
pectinpolygalacturonase, *2*: 1098
peeling, *2*: 1097
quality standards, *2*: 1099
sorting and grading, *2*: 1096
thermal processing, *2*: 1099
Canned tuna, *1*: 19, *2*: 953
Canned, frozen, and preserved fruits, vegetables, *1*: 94
Canning, *1*: 857
Capelin, *2*: 932
Cappuccino, *2*: 404–406
Capric acid, *1*: 736, *2*: 383
Caprine, *2*: 387
Caprino del Piemonte, *2*: 505–506, 509
Caproic acid, *2*: 383, 732
Caprylic acid, *2*: 383
Capsaicin, *1*: 212, 217
Capsicum, *1*: 217
Captopril, *2*: 502–503
Caramel, *2*: 404–406
Caraway, *1*: 210
Carbohydrates, *1*: 1001, 1004, *2*: 101, 105, 257, 266, 284–285, 287–288, 468, 476, 688
fermentable carbohydrates, *1*: 920
Carbon dioxide (gas carbonium), *1*: 420
analytical procedure for bottler's carbon dioxide, *1*: 433
feed gas sources, *1*: 433
purification, *1*: 433
quality guidelines for bottler's carbon dioxide, *1*: 433
quality verification levels, *1*: 433
safety, *1*: 434
storage, *1*: 434
Carbon dioxide, *1*: 446, 471, 479, 482, *2*: 478
Carbonated soft drinks (CSDs, soft drinks, soda pops)
facts and figures, *1*: 421
history, *1*: 420
process of manufacturing, *1*: 422
concentrates, *1*: 533–534
filling, sealing, and packing, *1*: 437
finished product, *1*: 440–441
overall process, *1*: 271, 422
quality control and assurance, *1*: 439–440
sweeteners, *1*: 423, 429
syrup preparation, *1*: 434
water, *1*: 425
Carbonation, *1*: 421–422, 436–437, *2*: 406
Bunsen coefficient, *1*: 422
carbonating technology, *1*: 437
control, *1*: 436
favorable results, *1*: 421
gas volume, *1*: 422
process, *1*: 421
reactions, *1*: 421
Carbonyl sulfide, *2*: 724
Carbonyls, *1*: 186
Carboxylic acids, *1*: 210
Cardamom, *1*: 167, 213
Cardiac function, *2*: 469
Cardiovascular diseases, *2*: 46
Carnitine, *2*: 458
Carotenoids, *1*: 934, 998, 1005, *2*: 43–44, 56, 58, 61, 976, 1112
bioavailability, *2*: 60
β-carotene, *2*: 61
tomato, *2*: 59
Carrageenan, *2*: 404–405, 605
Carrier materials, *1*: 486–487
selection criteria, *1*: 486
Carrot, *1*: 219
Carrots, frozen, *2*: 988–989
styles, *2*: 988
chips, *2*: 989
cut, *2*: 989
halves, *2*: 989
quarters, *2*: 989
sliced, *2*: 989

Carrots, frozen (*Continued*)
strips, *2*: 989
wholes, *2*: 989
Carveol, *1*: 187
Carvone, *1*: 210
Caryophyllene, *1*: 858
Case hardening, *1*: 869, *2*: 1113
Casein micelles, *1*: 648, 652, 974
Caseins (CN), *1*: 579, 581–582, 584, 589–590, *2*: 380, 386–388, 391–393, 464, 471, 558–559, 695, 705
α-, *2*: 464
αs1-, *2*: 464, 471–472
αs2-, *2*: 464
β-, *2*: 464
κ-, *2*: 464
acid casein, *2*: 567
alleles, *2*: 472
applications, *2*: 570
associative state, *2*: 560
casein co-precipitates, *2*: 564
casein fractions, *2*: 560, 569
casein micelle, *2*: 570
caseinates, *2*: 569
exports and imports, *2*: 560
genotype, *2*: 474
global production, *2*: 558
hydrolysates, *2*: 562
loci, *2*: 464
micellar casein, *2*: 568
micelles, *2*: 472
molecular properties, *2*: 559
polymorphisms, *2*: 458
rennet casein, *2*: 564
stabilizer interaction, *2*: 653
yogurt gel strength, *2*: 652, 660, 664
Casein, bioactive peptides derived from, *2*: 109
Casein, functional peptides derived from, *1*: 974
Caseinoglycopeptide, *2*: 49
Caseinomacropeptide, *2*: 504
Caseinophosphopeptides, *2*: 493–495, 510
Casing, *2*: 252, 257–258, 261, 304
Casocidin, *2*: 500, 504–505
Casokinins, *2*: 490, 495, 497–499, 502, 509
Casomorphins, *2*: 490–493, 495–496, 499, 503, 509
Casopiastrin, *2*: 496
Casoplatelin, *2*: 496, 509
Cassava, *1*: 186
Cat's ear noodles, *1*: 544
Catabolite inactivation, *1*: 469
Catalase, *2*: 267–268
Catalytic
reaction, *2*: 465
Catechins, *1*: 918
Catfish, North American freshwater, *2*: 849–851
bone classifications, *2*: 850
definitions, *2*: 851
description, *2*: 849
market forms, *2*: 850
styles, *2*: 850
types, *2*: 850
Cathepsins, *2*: 239, 723, 756
Cauliflower, frozen, *2*: 989
styles, *2*: 989
clusters, *2*: 989
nuggets, *2*: 989
CCP, *1*: 238
Celiac disease, *1*: 1018–1020, 1022
clinical symptoms, *1*: 1019
diagnostic procedures, *1*: 1018
epidemiology, *1*: 1019
iceberg model, *1*: 1019
possible causes, *1*: 1019
Cell, *1*: 289, 291
size, *1*: 289, 293
uniformity, *1*: 293
walls, *1*: 293
Cell differentiation, *2*: 49
Cell envelope-associated proteinases, *2*: 723
Cell proliferation, *2*: 44–48
Cell signalling, *2*: 44, 46, 49
Cellophane noodles, *1*: 559
Cellulases, *1*: 188
Centrifugation, *1*: 162
CEP. *2*: *See* Cell envelope-associated proteinases
Ceratocystis, *1*: 185
Ceratocystis fimbriata, *1*: 194, 196–197, 200, 202
Ceratocystis moniliformis, *1*: 187, 202
Ceratocystis variospora, *1*: 187
Cereal breakfast foods, *1*: 99
CFR, *1*: 225

CGMP, *2*: 950, 952
CGMPR, *1*: 224, 243, *2*: 950
Cheddar, *1*: 582–590, *2*: 394, 499, 501, 503
Cheddar cheese manufacturing, *1*: 54, 56
Cheese, *1*: 48–51, 652, *2*: 387, 391, 450, 476
  approximate weight, *1*: 53
  basic cheese-making steps, *1*: 54
  brown whey, *2*: 478
  cheddar, *2*: 464
  classifications, *1*: 52
  packaging material requirements, *1*: 53
  yield, *2*: 470, 472
Cheese types
  American cheese, *1*: 596, 600
  Colby, *1*: 569, 572–573, 575, 582, 586
  cold-pack cheese food, *1*: 596–597, 599
  cold-pack cheese spread, *1*: 600
  cottage, *1*: 598–599, 618–619
  Jack. *See* Monterey
  Longhorn, *1*: 569
  Monterey, *1*: 569–570, 582, 584
  pasteurized process cheese, *1*: 596–603, 606, 608, 610, 612, 614
  pasteurized process cheese product, *1*: 599–600, 611
  pasteurized process cheese spread, *1*: 597, 599, 606
Chemical anchors for specific flavors or off-flavors, *2*: 733
Chemical
  characteristics, *2*: 461
  composition, *2*: 449
    general, *2*: 458
  structure, *2*: 461
Chemical composition, *2*: 858
Chemical composition of milk, *1*: 972, *2*: 348
Chervil, *1*: 209
Cheshire, *2*: 719
Chewing gum, *1*: 106
Chhana, *1*: 638–639, *2*: 478
  manufacture, *1*: 639
Chiffon cake, *1*: 318
Chili, *1*: 219
Chili pepper, *1*: 212
Chilling, *2*: 478
Chilling and refrigeration processes, *1*: 27–28
Chinese Jinghua ham processing, *1*: 64
Chinese pickled vegetables, *1*: 79–80
  basic steps, *1*: 80
Chinonectes spp., *2*: 897
Chitin, *1*: 891, *2*: 908
Chitosan, *2*: 908
Chives, *1*: 209
Chloride, *2*: 731
Chloride salts, *2*: 920
Chlorination, flour, *1*: 309
Chocolate, *1*: 919, *2*: 404–409, 617, 640, 641
  bloom, *1*: 690
  conching, *1*: 683
  depositing, *1*: 687
  enrobing, *1*: 686
  liquor, *1*: 676
  manufacturing, *1*: 682–686
  molding, *1*: 687–688
  nutrition, *1*: 691–693
  rancidity, *1*: 691
  refining, *1*: 682–683
  storage, *1*: 691
  sugar-free, *1*: 689–690
  tempering, *1*: 684–686
  U.S. standards, *1*: 680–681
  viscosity, *1*: 683–684
Chocolate and cocoa products, *1*: 105–106
Chocolate and cocoa products establishment inspection, *1*: 696–698
  processing, *1*: 697
  raw materials, *1*: 696–697
  sample collection, *1*: 697–698
Chocolate coating
  compound, *1*: 688–689
  sugar free, *1*: 689–690
Chocolate mix, *2*: 479
Cholecalciferol, *2*: 47
Cholesterol, *1*: 189, 719, *2*: 273, 461
  deposition, *2*: 480
  dissolution, *2*: 480
  esterified, *2*: 463
  esters, *2*: 462–463
  free state, *2*: 461
  metabolism, *2*: 480
Chronic enteropathy, *2*: 479
Churning butter, *2*: 478
Chylomicron, *2*: 383
Chyluria, *2*: 480

Chymosin (also rennet), *1*: 572, 578, *2*: 495–496, 504, 506–507, 723–724
Chymotripsin, *2*: 494, 502
Cinnamaldehyde, *1*: 210
Cinnamic aldehyde, *1*: 211
Cinnamon, *1*: 211, 213–214
CIP, *2*: 241
Cis-3-hexenal, *1*: 857, 858
Cis-4-heptenal, *2*: 938
Citral, *1*: 187, 853, 858
Citrate (or citric acid), *2*: 388, 637, 644
Citrate, *1*: 476, 728
Citrate metabolism, *2*: 719
Citric acid, *1*: 604, 853, *2*: 404–405, 462
Citronellal, *1*: 167, 187
Citronellol, *1*: 187
Citrus
  flavonoids, *1*: 918–919
  fruits, *1*: 917
  limonoids, *1*: 917
Citrus juice, *1*: 845–860
Clams
  hard clam (quahog), *2*: 898
  processing, *2*: 905
  sea (skimmer) clam, *2*: 895
  soft clam, *2*: 898
  surf clam, *2*: 898
Clarification, *2*: 478
Cleaning, *1*: 21
Cleanliness, *1*: 215–216, 226
Clevenger apparatus, *1*: 149
Climate, *2*: 452
Clinical nutrition products, *1*: 969
Clostridium botulinum, *2*: 341
Clostridium butyricum, *1*: 197
Clotting factors, *2*: 49
Clove, *1*: 213
Clumping. *See* Partial coalescence
Coagulation, *1*: 286, 456–457, 463, *2*: 472
  properties, *2*: 470
  slow, *2*: 476
Coagulating ability, *2*: 472
Coalescence, *1*: 658
Coating(s), *2*: 639, 641
Cocoa, *2*: 404–405, 479, 637
  powder, *1*: 678–682
Cocoa beans, *1*: 696
  filth, *1*: 696
  fumigation, *1*: 696
  insect infestation, *1*: 696
  inspection, *1*: 696
  microbial tetsing, *1*: 696
  moldy beans, *1*: 696
  preliminary field examination, *1*: 696
  Salmonella Free Certificate, *1*: 697
Cod, *2*: 858, 874–876, 878–879, 884–885, 932, 935, 942
Code of federal regulations, *2*: 595
Codex Alimentarious, *1*: 236–237, 283, 1021
Codex Alimentarius Commission, *1*: 136, *2*: 1009–1010
  HACCP, *2*: 1009
  quality, *2*: 1010
  retail, *2*: 1010
  safety, *2*: 1010
Codex classification, *1*: 207
Coefficient α, *2*: 861, 862
Coffee, *2*: 404, 408
Cold liquid freezers, *1*: 31
Cold press, *1*: 211
Cold pressing, *1*: 152
Cold shortening problem, *2*: 877
Cold-trap on-line sampling method, *1*: 396–400
Coliform bacteria, *2*: 457
Colligative properties, *2*: 376
Colloidal stability, *1*: 451, 457, 466, 479
Colon cancer, *1*: 920
Color, *1*: 569, 573, 576–577, *2*: 237, 244–245, 252, 256, 261–262, 267, 308, 315, 366, 1051, 1055, 1061
Color additives, *1*: 35
Color additives status list (CASL), *1*: 253
Color values, *1*: 217
Colostrum, *1*: 739
Comminution, *2*: 258
Commonality, *2*: 465
Competition for substrates, *2*: 155
Complementary and alternative medicine (CAM), *2*: 177
Composite, *1*: 656
Composition, *2*: 449, 459
  cow's milk, *1*: 47, 972
  wheat, flour, and germ, *1*: 67
Composition of milk, *2*: 348
Compressor, *2*: 872

Comte, *2*: 739
Concentrate, *2*: 456, 479
  diet, *2*: 459
  feed, *2*: 456
Conching of chocolate, *1*: 683
Concomitantly, *2*: 461
Condensed milk, *2*: 602
  superheated, *2*: 603
Condensed products, *2*: 474
Condenser, *2*: 479
Conditioned, *2*: 1015
Conditioning, *2*: 1015–1016
Conduction heat, *1*: 322
Conformation, *2*: 465
Conjugated linoleic acid (CLA), *1*: 921, *2*: 106, 383, 463
Consistency, *2*: 477
Constituent, *2*: 467
Consumer acceptance, *2*: 882
Consumer Health Information for Better Nutrition Initiative of the U.S. Government, *1*: 929
Consumer taste, *2*: 450
Consumer use, *1*: 665
Consumption, *1*: 596, 611, *2*: 451, 454
Containers, *2*: 859, 869
Contaminants, *2*: 953, 959
Contamination, *2*: 859, 868, 870, 873, 880–881, 887
Continuous mixers, *1*: 320
Continuous subcritical water extraction, *1*: 152, 154
Control of foreign objects, *1*: 698
Controlled atmospheric storage (CAS), *1*: 28
Convalescent treatment, *2*: 580
Convection heat, *1*: 322
Convection oven, *1*: 322
Conventional method, *1*: 314
Conventional meringue method, *1*: 315
Cooked ham, *2*: 241, 249, 286, 291, 296, 312–315
  cooling, *2*: 244
  massaging, *2*: 243
  reception of hams, *2*: 241–242
  resting, *2*: 243
  smoking, *2*: 244
  tumbling, *2*: 243
Cookies and crackers, *1*: 103
Cooking, *1*: 579–581, 585, 598, 607, 622
Cooling, *1*: 261, 266, 270, 274
  air blast cooler, *1*: 270
  natural cooling, *1*: 270
  vacuum cooling, *1*: 270
Cooperative, *2*: 454
Co-PET, *1*: 860
Copper, *2*: 390, 458
Corn, *1*: 370
  dry milling, *1*: 373
  gluten-free pasta, *1*: 376
  milling fractions, *1*: 373
  polenta, *1*: 374
  wet milling, *1*: 372
Corn on the cob, frozen, *2*: 989–980
  colors, *2*: 990
  lengths, *2*: 990
  styles, *2*: 989
    natural, *2*: 989
    trimmed, *2*: 989
Corn syrup, *2*: 479, 603
Coronary bypass, *2*: 480
Cottage cheese, *2*: 334
Cottage cheese manufacturing, *1*: 54–55
Cottage industry, *2*: 476
Cottonseed oil mills, *1*: 106
Cow, *2*: 380, 383–387, 389–391, 393–395
Cow breed
  Holstein, *2*: 460, 480
  Jersey, *2*: 460, 480
Cowy flavor, *2*: 733
Coxiella burnetti, *2*: 336
Crabs, *2*: 882–883, 932–942
  Alaska king crab, *2*: 897
  blue crab, *2*: 897
  blueing, *2*: 905
  crabmeat processing, *2*: 897
  Dungeness crab, *2*: 897
  mushiness, *2*: 905
  stone crab, *2*: 897
  swimming crab, *2*: 897
  Tanner crab, *2*: 897
  walking crab, *2*: 897
Crabtree effect, *1*: 471
Cragon franciscorum augustimana, *2*: 895
Cranberries, *1*: 917
  nondialysable polymeric compound, *1*: 917

Crassostrea
gigas, *2*: 898
virginica, *2*: 898
Cream, *1*: 652–653
age gelation, *1*: 743
agglutinin reaction, *1*: 735, 739
air cells, *1*: 734, 741–742
churning, *1*: 742
composition, *1*: 726–727
creaming, *1*: 735
feathering, *1*: 743
frozen, *1*: 738
heat stability, *1*: 729
Kreiger–Dougherty equation, *1*: 736
lipolysis, *1*: 732–733
off-aroma, removal of, *1*: 741
Ostwald ripening, *1*: 741
over-run, *1*: 740–741
pasteurization, *1*: 727–728, 733, 740
re-bodying effect, *1*: 736, 738–739
recombined, *1*: 737
rheology, *1*: 683, 687
separation, *1*: 728
spinning cone technology, *1*: 745
stabilizers, *1*: 737, 741, 743
Stokes equation, *1*: 735
vacreation, *1*: 745
volatiles, *1*: 745
Cream cheese, *1*: 652–667
Cream dressing (also dressing, also creaming), *1*: 618, 620–621, 625, 627–628
Cream of Tartar, *1*: 313, 317
Cream, types of
clotted, *1*: 739
double, *1*: 739
dried, *1*: 740
half and half, *1*: 739, 742
heavy, *1*: 739
light, *1*: 739
liqueur, *1*: 744
plastic, *1*: 736
sterilized, *1*: 742
ultra-high temperature (UHT) treated, *1*: 733
whipping, *1*: 740–742
Creamery butter, *1*: 91
Creaming, *1*: 312
Creaming method, *1*: 314
Creatin, *2*: 467
Creatinine, *2*: 467
Crescenza, *2*: 499, 501, 503, 506, 509
Critical control point (CCP), *1*: 225, 236–239, 242, *2*: 948
Croissant, *1*: 70
Crossbreeding, *2*: 454
Cross-linkages
intermolecular, *2*: 879
Cruciferous vegetables, *1*: 916–917
Crumb, *1*: 300
Crust, *1*: 287, 292
blisters, *1*: 298
color, *1*: 288, 292, 298
Crustaceans, *2*: 932–942
Cryogenic cooling, *1*: 27
Cryogenic freezers, *1*: 31
Cryoglobulins, *1*: 738–739
Crystal(s), *2*: 394, 637–638
ice crystals, *2*: 637–641
Crystallization
ice, *2*: 860, 862, 864
CSWE. *See* Continuous subcritical water extraction
Cu mien (thick noodles), *1*: 543
Cucumbers, *2*: 1046
Culinary herbs, *1*: 122
Cultured cream, *2*: 415, 444–446
Cultures, *1*: 569–570, 572, 574, 576, 578, 587–588, 619, 622, *2*: 476
probiotic, *1*: 985, *2*: 102
yogurt, *1*: 984, *2*: 102
Cumin, *1*: 213
Curcumene, *1*: 210
Curcumin, *1*: 217
Curd, *2*: 391
Curd tension, *2*: 375, 475, 477
Curing salt, *2*: 236, 252
Current good manufacturing practice(s) regulations (CGMPR), *1*: 224, *2*: 950
Cutting, *1*: 579–580
Cymbopogon winterianus, *1*: 167
Cystic fibrosis, *2*: 480
Cytomodulatory peptides, *2*: 495
Cytoplasm, *2*: 382

2,5-Dimethyl-4-hydroxy-3(2H)-furanone, *1*: 855
2,3-Decadienal, *2*: 940
2,5-Dimethylpyrazine, *1*: 392, 402
4-Decalactone, *1*: 187, 196
7-Dehydrocholesterol, *2*: 47–48
β-Damascenone, *1*: 855, 861
γ-Decalactone, *1*: 172, 201
®-δ-Dodecanolide, *1*: 196
Dai mien, *1*: 544
Dairy, *2*: 450–451
  cows, *2*: 451
  goats, *2*: 451–452
    farming, *2*: 450
    industry, *2*: 450, 454
  products, *2*: 450
  sheep, *2*: 450
    farming, *2*: 450
  species, *2*: 450, 456
Dairy products, *1*: 920
Dang myun, *1*: 561
Danish pastries, *1*: 70–71
D-carvone, *1*: 210
De novo, *2*: 383
Debaromyces, *1*: 181
Decanal, *1*: 857
Decarboxylation, *2*: 268, 270, 288–289
Decoction, *1*: 447, 451–452, 455, 458
Decomposition, *2*: 949–952, 954, 959–960
Decreamed, *2*: 478
Defects, *2*: 1055
Deficiency, *2*: 40–41
  vitamin A, *2*: 44
  vitamin D, *2*: 47–49
  vitamin E, *2*: 45–46
  vitamin K, *2*: 50–51
Definition of milk, *2*: 349
Degrees Baumé, *2*: 1050–1055
Dehydration, *2*: 823–826, 830, 859, 866–868, 887
  osmotic, *2*: 1114
  tomato, *2*: 1110
Deletion, *2*: 470
Denaturation
  freeze, *2*: 888
  protein, *2*: 857–858, 877–880, 883
Density, *2*: 373
Depositing batter, *1*: 321
  types, *1*: 321
Depression, *2*: 459
Derived flavor, *1*: 121
Descriptive sensory analysis, *2*: 731
Desertification, *2*: 452
Destabilization, *2*: 357–358, 474, 879
Detoxify, *2*: 467
Dextrins, *1*: 450, 468
Dextrose equivalent, *2*: 479
DFD meat, *2*: 235, 241, 322
DHDA. *2*: *See* Dynamic headspace dilution analysis
Diabetes, *2*: 177–179, 185, 188–189
Diacetyl, *1*: 175, 182, 471, 477–481, 485, 488–490
Diameter, *2*: 461
Diced tomatoes, *2*: 1100
  quality standards, *2*: 1100
  size reduction, *2*: 1100
Diet, *2*: 468–469
  low-fat, *2*: 478
Dietary approaches to stopping hypertension (DASH), *2*: 210
Dietary fiber, *1*: 1004, 1015, 1020
  insulin, *1*: 1023
Dietary manipulation, *2*: 463
Dietary nutrient
  goals, *1*: 991
  intake, *1*: 991
Dietary supplements, *1*: 940
  Canada, *1*: 941
  Europe, *1*: 941
  Japan, *1*: 941
  United States, *1*: 941
Digestibility, *2*: 479
Diglycerides, *1*: 212, 710
Dilactone, *1*: 853
Dill oil, *1*: 210
Dimethyl disulfide, *2*: 724, 731, 941
Dimethyl sulfide, *2*: 724
Dimethyl tetrasulfide, *2*: 724
Dimethyl trisulfide, *2*: 724, 941
Dimethylamine (DMA), *2*: 858, 877, 881, 884, 942
Dimethylanthramilate, *1*: 853
Dipeptidylpeptidases. *See* Peptidases
Direct dryer, *1*: 164
Directly acidified cheeses, *1*: 648
Discoloration, *2*: 824–825, 827, 829, 845
Disease, *1*: 990–991, 997, 1001

Disintegrating, *1*: 22
Disodium 5′-guanylate, *1*: 123, 129
Disodium 5′-inosinate, *1*: 123, 129
Distillation, *1*: 149, 157–162
Distillation columns, *1*: 158
Distilled and blended liquors, *1*: 109–110
Distribution, *2*: 480
Disulfide bonds, *2*: 465
D-limonene, *1*: 860
DMA. *2*: *See* Dimethyl amine
DMDS. *2*: *See* Dimethyl disulfide
DMHF. *2*: *See* 4-Hydroxy-2,5-dimethyl-3(2H)-furanone
DMS. *2*: *See* Dimethyl sulfide
DMTS. *2*: *See* Dimethyl trisulfide
DNA, *2*: 470
  fragment, *2*: 472
  rearrangement, *2*: 472
Dog and cat food, *1*: 101
Double acting baking powder, *1*: 312
Douchi, *1*: 73
Dough making, *1*: 265, 267–268, 274
  energy input, *1*: 267
  flour hydration, *1*: 267
  mixing, *1*: 263, 266–267, 271– 273
  rheological characteristics, *1*: 267
  water addition, *1*: 265–266
Dr Brian Lawrence's bible, *1*: 211
Drained weight/volume, *2*: 1054
Draining, *1*: 576, 582, *2*: 477
Dried and dehydrated fruits, vegetables, and soup mixes, *1*: 96
Drier, *1*: 870
  convective air, *1*: 870
  solar, *2*: 1115
  tunnel, *1*: 870, *2*: 1116
Drip, *2*: 857, 864, 876–878, 882–883, 906
Dry, condensed, and evaporated dairy products, *1*: 92–93
Dry cured ham manufacturing, *1*: 63–64
Dry-cured ham, *2*: 233–249, 282, 285–286, 290–294, 304, 316–323
  accelerated processing, *2*: 241
  boned, *2*: 237
  drying, *2*: 237, 322
  post-salting, *2*: 234, 237
  pre-salting, *2*: 236
  reception of hams, *2*: 234–236
  ripening, *2*: 237
  smoking, *2*: 237
  traditional processing, *2*: 234, 241
  types of
    Bayonne, *2*: 240
    Corsican, *2*: 240, 323
    country-style, *2*: 236, 241, 323
    French, *2*: 222, 236, 240
    Iberian, *2*: 237, 240, 292, 323
    Italian, *2*: 240
    Katenschinken, *2*: 241
    Kentucky, *2*: 241
    Parma, *2*: 236, 240, 292
    San Danielle, *2*: 240
    Serrano, *2*: 323
    Spanish, *2*: 236, 240, 292
    Virginia, *2*: 241
    Westphalia, *2*: 241
    Yin Hua, *2*: 241
    Yunnan, *2*: 241
Drying, *1*: 162–167, 867–869, *2*: 404, 406, 477
  convective, *2*: 1116
  roller, *2*: 479
  spray, *2*: 477, 479, 1117–1118
Drying effects, *1*: 166–167
  freeze, 872
  microwave, *1*: 871
  solar, *1*: 869
  sun, *1*: 869
D-tagatose, *1*: 923
Dynamic headspace dilution analysis, *2*: 731

2-Ethyl-3,5-dimethylpyrazine, *2*: 731
4-Ethyl octanoic acid, *2*: 731
4-Ethyloctanoate, *2*: 463–464
(E)-4,5-epoxy-(E)-2-decenal, *2*: 726
(−) Epicatechin, *1*: 919
Echinacea, *1*: 925
Economy, *2*: 450
  national, *2*: 450
Eczema, *2*: 166
Edam, *2*: 502
Edible coatings, *1*: 890–891
  lipid, *1*: 890
  polysaccharides, *1*: 890–891
    alginates, *1*: 890
    casein, *1*: 890
    celluloses, *1*: 890

chitosan, *1*: 890–891
pectin, *1*: 890
proteins, *1*: 890
Edible films and coatings, *2*: 799
active packaging, *2*: 807–808
antimicrobial agents, *2*: 799–808
antimicrobial packaging, *2*: 808
antioxidants, *2*: 807–808
applications, *2*: 800–804
carbon dioxide generating systems, *2*: 807
composite films, *2*: 804
definition, *2*: 799
ethanol generating systems, *2*: 807
formation mechanisms, *2*: 805
complex coacervation, *2*: 805
gelation, *2*: 805
simple coacervation, *2*: 805
history, *2*: 799
importance, *2*: 799–800
lipid based, *2*: 800–801
glycerides, *2*: 801
waxes, *2*: 801
manufacture, *2*: 805
methods for application, *2*: 806
plasticizers, *2*: 805
polysaccharide based, *2*: 801
agar, *2*: 802
alginates, *2*: 801–802
carrageenan, *2*: 802
cellulose and cellulose derivates, *2*: 803
chitosan, *2*: 802–803
starch, *2*: 802
protein based, *2*: 803–804
corn zein, *2*: 803–804
gelatin, *2*: 803
soy protein isolate, *2*: 804
whey protein isolate, *2*: 804
Education and training, *1*: 227
Effectiveness, *2*: 468
Effects of heat, *2*: 371
Egg noodles, *1*: 546–549
Egg products, *2*: 606
Egg yolk solids, *2*: 479
Eggs, *1*: 289, 312–313
foam, *1*: 312
substitutes, *1*: 313
whites, *1*: 313
yolks, *1*: 313
Eland, *2*: 380
Electrical conductivity, *2*: 370
Electrochemical properties, *2*: 370
Electrophoresis, *2*: 470
Electrophoretic mobility, *2*: 465
Elettaria cardamomum, *1*: 167
Embden–Meyerhof–Parnas, *2*: 101
Embden–Meyerhof–Parnas pathway, *1*: 181, 471
EMC. *See* Enzyme-modified cheese
E-min, *1*: 550
Emmental, *1*: 596, 605, *2*: 502
Emulsifier(s), *1*: 311, *2*: 479, 596, 604, 606, 643
Emulsifying agents, *1*: 596, 601–604, 606–608, 613
citrate(s), *1*: 601–602, 607
phosphate(s) (mono, ortho, poly, pryo), *1*: 601–603, 607–608
trisodium citrate, *1*: 603, 607
trisodium phosphate, *1*: 602
Emulsion, *1*: 603, 605–606, 610, 655–656
Enalapril, *2*: 501
Encapsulated flavorings, *1*: 125–126
Encephalopathy, *2*: 168
Endonucleases, *2*: 471
Endopeptidases, *2*: 933–935
Energy, *2*: 454, 480, 873, 875–876, 877
Enfleurage, *1*: 148, 149, 155
Enhancer, *1*: 128
Enkephalins, *2*: 491, 497
Enterocins, *2*: 91, 93
Enterococcus faecium, *2*: 509
Enterococcus spp., *2*: 86, 91–92, 94
Enterococcus faecalis, *2*: 86
Enterococcus faecium, *2*: 86
Enthalpy, *2*: 861
Environment
conditions, *2*: 459
tropical, *2*: 460
Enzymatic reactions, *2*: 239–240, 245–246
Enzymatic technology, *1*: 188–189
Enzyme inactivation, *2*: 220, 243
Enzyme(s), *1*: 569–573, 575, 578, 580, 584, 587, 600–601, 613, 620, 623, 726–727, 732, *2*: 387, 467, 471
alkaline phosphatase, *2*: 468

Enzyme(s) (*Continued*)
glutathione peroxidase, *2*: 469
lactic dehydrogenase, *2*: 468
lipase, *2*: 463
lysozyme, *2*: 467
malic dehydrogenase, *2*: 468
protease, *2*: 467
ribonuclease, *2*: 467
xanthine oxidase, *2*: 468
Enzyme-modified cheese, *1*: 188–189
EO. *See* Essential oils
Ephedra (Ma huang, herbal ectasy, mahuanggen), *1*: 925
Epilepsy, *2*: 480
Epithelial cell, *2*: 389
Epithelial surface, *2*: 156
competition, *2*: 156–157
adherence, *2*: 157
receptor, *2*: 156
Equalization, *2*: 1050
Ergosterol, *1*: 474, 482, *2*: 47–48
Erwinia caratovora, *1*: 186
Erythritol, *2*: 639
ESA. *See* European Spice Association
Escerichia coli, *2*: 490, 504–505, 507–509
Essential oils, *1*: 148, 160–162, 180, 205–220
Ester linkage, *2*: 480
Esterases. *See* Lipases
Esters, *1*: 181, 183–186, 210, 471, 473–474, 476, 488, 852
acetate, *1*: 475
biosynthesis, *1*: 476
C_6–C_{10} medium chain fatty acid ethyl, *1*: 473
synthetase, *1*: 480
Estragole, *1*: 210
Ethanol acetyltransferase, *1*: 475
Ethanol, *1*: 444, 470, 489
Ethyl 2-methylbutanoate, *1*: 848, 852–853
Ethyl acetate, *1*: 182–186, 197, 212, 474–475, 485, 858
Ethyl acetoacetate, *1*: 392
Ethyl butanoate, *1*: 852–858
Ethyl butyrate, *1*: 183, 186, 780
Ethyl caproate, *1*: 474
Ethyl caprylate, *1*: 475
Ethyl furaneol, *2*: 731
Ethyl hexanoate, *1*: 474, 853, 861
Ethyl octanoate, *1*: 474
Ethyl propionate, *1*: 185, 853–854
Ethyl *trans*-2-*cis*-4-decadienoate, *1*: 854
Ethyl-2-octenoate, *1*: 854
Ethyl-4-decenoate, *1*: 854
Ethylene oxide, *1*: 217
Ethylene vinyl alcohol co-polymer, *1*: 859–860
ETO. *See* Ethylene oxide
Eugenol, *1*: 210
European Spice Association, *1*: 214
Evaluation (also defects), *1*: 584, 628
Evaporated, *2*: 474
Evaporation, *2*: 479
chamber, *2*: 479
Evaporation and drying, *1*: 31–32
Evaporative cooling, *1*: 27, 410–411
EVOH. *See* Ethylene vinyl alcohol co-polymer
Ewe, *2*: 476
Exclusion chromatography, *1*: 153
Exonic point deletion, *2*: 470
Exopeptidases, *2*: 932–934
Exopolysaccharide, *2*: 654, 660, 666
Exopolysaccharides (EPS), *2*: 416, 440
Exorphins, *2*: 491–492
Exotic types, *2*: 476
Extraction modes, *1*: 147–155
Extraction using microwaves, *1*: 153
Extraction with hot fat, *1*: 149, 155
Extraction with organic solvents, *1*: 149–150
Extraction with sonic waves, *1*: 153
Extraction with supercritical carbon dioxide, *1*: 150–152
Extractive distillation, *1*: 154, 159
Extraneous vegetable material (EVM), *2*: 1052
Extruded (also extrusion, and cold extrusion), *2*: 641–644
Exudation. *See* Syneresis

Fabrication, *2*: 476
Factors, *2*: 452
FAO, *2*: 450–451, 465
WHO, *2*: 465
yearbook, *2*: 450
FAO/WHO, *1*: 283
Farmstead, *2*: 477
Farmstead cheese makers, *2*: 454

Fat, *1*: 287, 296, 596, 598, 604, 618, 659, 990–991, 993, 996–1001, 1003–1004, 1008, *2*: 381, 385, 389, 456, 637, 642–643. *See also* Lipids
emulsified, *1*: 311
fatness, *1*: 996
globule, *2*: 458, 461
size, *2*: 460, 461
hydrogenated, *1*: 302
low, *1*: 993, 997–998, 1000, 1006
oil, *1*: 296, 311
shortening, *1*: 296, 311
reduction, *1*: 990–991, 993, 995, 998, 1000
replacements, *1*: 1001, 1004
substitutes, *1*: 287, 1001
trans, *1*: 302
Fat globules
size, *1*: 727, 730–731
stability, *1*: 729–730, 733–735
surface load, *1*: 729
surface tension, *1*: 730, 733, 738
zeta potential, *1*: 727
Fat replacer(s), *1*: 288, 300
carbohydrate based, *1*: 288
lipid based, *1*: 281
Fats and oils, *1*: 106–108
Fatty acid, *2*: 239–241, 245–246, 256, 262, 272–273, 283–285, 308, 380–383, 447, 461, 463, 818, 877–878, 880, 886
branched-chain (BCFA), *2*: 463
capric, *2*: 463
caproic, *2*: 463
caprylic, *2*: 463
composition, *2*: 443, 464
essential, *2*: 465
lauric, *2*: 463
medium chain (MCT), *2*: 463, 480
methyl esters, *2*: 463
monomethylated, *2*: 463
oleic, *2*: 463
palmitic, *2*: 463, 480
short chain, *2*: 464, 472
unsaturated, *2*: 472
volatile, *2*: 472
insoluble, *2*: 472
soluble, *2*: 472
Fatty acids, *1*: 467, 471, 474, 482
unsaturated, *1*: 474, 482
Fatty acids metabolism, *2*: 722
FDA, *1*: 224, 233, 240, 300, *2*: 948–950, 953–954
food code, *1*: 224, 240, 243
Feed off-flavors, *2*: 733
Feeding, *2*: 452
supplementary, *2*: 454
under, *2*: 454
Fen si, *1*: 559
Fennel, *1*: 213
Fennel oils, *1*: 210
Fermentation, *1*: 38, 180–181, 206, 663, *2*: 96, 98, 100, 102, 260, 265–274, 478
alcoholic, *2*: 478
continuous, *1*: 426, 428
high-gravity, *1*: 468–469, 474, 483
main/primary, *1*: 457–478
secondary, *1*: 479, 482, 485, 488
Fermentation process types, *1*: 196–200
Fermentation systems, *1*: 194–200
Fermented, *2*: 478
products, *2*: 474
Fermented cereal products, *1*: 66–71
ingredients, *1*: 66
types, *1*: 66
Fermented dairy products, *1*: 46–63
common organisms used, *1*: 50
dairy starter cultures, *1*: 51
ingredients, *1*: 46, 49
kinds, *1*: 46
temperature requirements, *1*: 52
types and examples, *1*: 48
Fermented foods
application of biotechnology in manufacturing, *1*: 80
process mechanization, *1*: 80–81
Fermented liquid milks, *1*: 59–63
Fermented meat products, *1*: 63
ingredients, *1*: 63–64
types, *1*: 63
Fermented milks, *2*: 411
claims, *2*: 100
classification, *2*: 412–413, 432
definition, *2*: 103
fermented milks with yeast and lactic starter cultures, *2*: 423–425

Fermented milks (*Continued*)
health benefits, *2*: 106
antiosteoporotic and antiobesity effects, *2*: 103
lactose intolerance, *2*: 102
physiological activity, *2*: 104
manufacturing, *2*: 413–426
nutritional aspects, *2*: 101
patterns of production in Europe, *2*: 413
process flowchart, *2*: 440
production, *2*: 97
regulatory aspects, *2*: 99
requirements for production, *2*: 434
milk homogenization, *2*: 437
milk pasteurization, *2*: 436
milk quality, *2*: 434
quality and functionality of other components, *2*: 434
types of culture, *2*: 434, 437
set-type products, *2*: 416
shelf life of product, *2*: 441
stirred-type products, *2*: 415
trends in Asia, *2*: 433
use of mesophilic bacterial strains, *2*: 415–417
use of probiotic bacterial strains, *2*: 422–425
use of thermophilic bacterial strains, *2*: 417–422
weight loss, *2*: 104
Fermented sausages. *See* Sausages
Fermented soy pastes, *1*: 74–75
Fermented soy products, *1*: 71–77
ingredients, *1*: 71, 72
types, *1*: 71
Fermented tofu, *1*: 75–76
Fermented vegetables, *1*: 77–80
kinds, *1*: 79
raw ingredients, *1*: 77
Fermented whole soybeans, *1*: 73–74
Ferulic acid, *1*: 861
Feta cheese manufacturing, *1*: 57, 59
Feverfew, *1*: 925
FFA. *See* Free fatty acids
Fiber, *1*: 982
Fibrinogen, *2*: 496
Fill of container, *2*: 1053
Fillet, *2*: 857, 861–864, 867, 870, 874–876
Filling, *1*: 665
Film-jolk, *2*: 432
Filtering, *2*: 477
Filth, *2*: 947, 949, 955, 958, 1058, 1060–1061
Finished product sampling, *1*: 697–698
Firmness, *2*: 472–473
curd, *2*: 472
Fish, *1*: 919
Asian seabass, *2*: 972, 978
Atlantic salmon, *2*: 970, 975
blood, *2*: 882
carp, *2*: 970–971
channel catfish, *2*: 970–971
composition, *2*: 858, 877, 885
discoloration, *2*: 882
fatty, *2*: 858, 877, 884–885
fillet, *2*: 861–864, 876
flavor of, *2*: 883–884
fresh, *2*: 856–857, 881, 883, 885
frozen, *2*: 857, 874, 878–879, 881, 887
glycogen content, *2*: 877
health, *2*: 972–973
lean, *2*: 858, 885
mackerel, *2*: 857, 885, 887
mince, *2*: 879, 883, 887
moisture, *2*: 863
muscle, *2*: 857, 859, 877–880, 883
number of myotomes, *2*: 858
nutrition, *2*: 973–974
odor of, *2*: 883
oxidation of, *2*: 882
property of, *2*: 878
protein, *2*: 883
quality, *2*: 856
rainbow trout, *2*: 970
size of, *2*: 858
species, *2*: 857–858, 877, 884–887
surface, *2*: 875, 882
texture, *2*: 883
tissue, *2*: 857, 879, 881, 885, 888
tuna, *2*: 977–978
types of skeletal muscle, *2*: 858
value-added, *2*: 857
white, *2*: 373
white muscle, *2*: 358

Fish and mince meat quality, *2*: 917
Fish, formulated, *2*: 916
  product thawing during retailing and home preparation, *2*: 926
  quality control during the processing, *2*: 920
  quality parameters of the formulated products, *2*: 916
  shelf-life, *2*: 926
Fish meal, *2*: 973
Fish oil, *2*: 973–974
Fish, processed, *2*: 916
Fish protein hydrolysates, *2*: 932–933
Flammulina velutipes, *1*: 183
Flanking, *2*: 472
Flat (layered) bread, *1*: 69–70
Flavonoids, *1*: 915, 917, *2*: 56–57, 59
  degradation, *2*: 58
  fruits, *2*: 58
  tomato, *2*: 58
Flavor, *1*: 169–174, 207, 293, 569–575, 581, 583–585, 601, 603, 605, 611, *2*: 237, 239–241, 244, 246, 252, 257, 261, 268, 271–273, 282, 285–296, 304–305, 312–323, 367, 474, 874, 881, 883–884, 887, 888
  alteration, *2*: 474
  cardboard, *1*: 481
  damage, *2*: 479
  fullness, *1*: 451, 479, 481
  ingredients, *2*: 476
  stability, *1*: 451, 457, 479
  threshold, *1*: 473, 476–477, 480
Flavor and odor, *2*: 831, 1052, 1055
Flavor chemists, *1*: 139
Flavor compounds
  biosynthesis, *1*: 183–187
  extraction, *1*: 200–202
  physical and chemical properties, *1*: 390
  produced by fungi, yeasts, and bacteria, *1*: 179–189
  thermal stability, *1*: 392–393
Flavor concentration
  on-line measurement apparatus, *1*: 391–392
Flavor extraction, *1*: 148–154
Flavor from fermented foods, *1*: 180–187
Flavor integers, *1*: 127
Flavor migration, *1*: 390–413
  in solid food matrices, *1*: 390–413
  system development, *1*: 391–393
Flavor migration in formulated dough, *1*: 400–408
Flavor perception, *1*: 127–129
Flavor production, *1*: 194
  effect of environmental conditions, *1*: 194
Flavor profile, *1*: 139
Flavor profile analysis, *1*: 143
Flavoring, *2*: 257, 260
Flavoring extracts and flavoring syrups, not elsewhere classified, *1*: 110–111
Flavorings produced by enzymatic hydrolysis, *2*: 931–940
Flavorist, *1*: 129, 139–140
Flavors, milk and mik products, *2*: 716
  characterization, *2*: 726
  chemistry, *2*: 733
  production, *2*: 716
  taste compounds, *2*: 736
Flavors, seafood byproducts, *2*: 932
Flaxseed, *1*: 915
Flocculation, *1*: 483–484
Flour, *1*: 265, 286, 295, 308–309
  all purpose flour, *1*: 286
  cake flour, *1*: 308
  self-rising flour, *1*: 309
  whole wheat, *1*: 286
Flour and other grain mill products, *1*: 98
Flour, common wheat, *1*: 332, 344, 355
  rheological characteristics, *1*: 332
Fluctuation, *2*: 468, 870–881, 885
Fluid milk, *1*: 93–94, *2*: 599
Foam stability, *1*: 458, 463, 466, 479
Foam style cakes, *1*: 317
  angel food cake, *1*: 317
  chiffon cake, *1*: 318
  Genoise cake, *1*: 318
  sponge cake, yellow, *1*: 318
Foaming, *1*: 438, *2*: 373, 404–405
Folate (folic acid), *2*: 154, 205, 467, 470
  binding protein, *2*: 467
Folding, *1*: 312
Food additives, *1*: 36
  adverse reaction monitoring system (ARMS), *1*: 36

Food additives (*Continued*)
approval process, *1*: 906
common uses in food categories, *1*: 34
Delaney clause, *1*: 36
direct food additives, *1*: 34
generally recognized as safe (GRAS), *1*: 35
indirect food additives, *1*: 34
regulations, *1*: 35–36
why, *1*: 33
Food Additives Amendment, *1*: 252
Food Allergen Labeling and Consumer Protection Act of, *1*: 2004, 284
Food alterations, *2*: 229
Food and Agricultural Organization (FAO), *1*: 11
Food and Agriculture Organization of the United Nations, *1*: 283
world Health Organization (FAO/WHO), *1*: 283
Food and color additives, *1*: 698–699
Food and drug administration (FDA), *1*: 246, *2*: 986
approved health claims, *2*: 11
Food and drugs act, *1*: 135
Food and Nutrition Board, *2*: 208, 211
Food Code, *1*: 224–242, *2*: 950
Food for special health use, *2*: 446
Food handling and processing, *1*: 10
Food labeling, *1*: 283
Food laws, *1*: 136
Food manufacturing (procesing) goals, *1*: 6
Food manufacturing plant
construction, design and maintenance, *1*: 248
environment, *1*: 247
plant services, *1*: 248
waste disposal, *1*: 248
Food odor research, *1*: 140
Food packaging and storage, *1*: 10
Food plant inspection, *1*: 246–252
equipment and utensils, *1*: 249–250
manufacturing process, *1*: 251–255
color additives, *1*: 253
food additives, *1*: 252
formulas, *1*: 251–252
ingredient handling, *1*: 251
packaging and labelling, *1*: 254–255
quality control, *1*: 253
personnel, *1*: 246–247
plants and grounds, *1*: 247–248
preparation, *1*: 246
raw materials, *1*: 249
handing procedures, *1*: 249
Food preparations, not elsewhere classified, *1*: 113–114
Food preservation, *1*: 10
Food spoilage
biological factors, *1*: 7
chemical (including biochemical) factors, *1*: 8
physical factors, *1*: 9
prevention and retardation, *1*: 10
sources of information, *1*: 11
Food standards, *1*: 252
Food Standards Agency of the United Kingdom, *1*: 283
Food Standards Australia, New Zealand, *1*: 283
Food technologist, *1*: 11
Foodborne disease
common bacteria genera, *1*: 8
most common pathogenic bacteria and viruses, *1*: 8
Foodborne infection, *1*: 8
Foodborne intoxication, *1*: 8
Foods for specified health use (FOSHU), *1*: 906
Formaldehyde (FA), *2*: 858
Forming, *1*: 23
confectionery, *1*: 23
meat and poultry patties, *1*: 23
pasta, *1*: 23
Formula, *1*: 288
Formulation, *1*: 143
Formulation of ice cream, *2*: 597
Fortification, *1*: 968
Fortified, *2*: 474
Fossa, *2*: 509
FPH. *See* Fish protein hydrolysates
Fractional distillation, *1*: 141
Fractionator, *1*: 159
Fractures, skeletal, *2*: 202, 209
Frankfurters, *1*: 12
Free amino acids, *2*: 858
Free fatty acids, (FFA), *1*: 9, 689, *2*: 463–464
Free moisture, *1*: 163
Free radicals, *1*: 858
Freeze-drying, *1*: 166

Freezer, *2*: 861–864, 868, 875
Freezing (and freezing point), *2*: 638–639, 641
  apparatus, *2*: 861
  biochemical aspects of, *2*: 857
  conditions, *2*: 881
  damage, *2*: 864, 885
  effect on quality, *2*: 859, 881
  equipment, *2*: 859–860, 863–866, 868, 873, 875
  medium, *2*: 867
  method, *2*: 863–864, 867
  physical point of view, *2*: 862
  physicochemical change, *2*: 876–877
  point, *2*: 860–863
  preservation, *2*: 857
  process, *2*: 861–862, 881
  rate, *2*: 860, 862–863, 865
  ratio, *2*: 863
  seafood, *2*: 877
  speed, *2*: 862, 864
  temperature, *2*: 860–861
  time, *2*: 860–864
Freezing and frozen storage, *1*: 29
  principles, *1*: 29
  quality changes, *1*: 29
  rate of freezing, *1*: 29
Freezing methods
  blast freezing, *2*: 899–900
  carbon dioxide freezing, *2*: 900–901
  cryogenic freezing, *2*: 900–901
  immersion freezing, *2*: 901–902
  liquid nitrogen freezing, *2*: 900
  plate freezing, *2*: 899
  pressure shift freezing, *2*: 902
Freezing point, *2*: 376
Freezing point depression, *2*: 604
Freezing process, *2*: 924
French paradox, *1*: 918
French vanilla, *2*: 479
Fresh and frozen shrimp, *2*: 840–842
  examination, *2*: 841
  market forms, *2*: 841
  styles, *2*: 840–841
  types, *2*: 840
Fresh cream, *2*: 600
Fresh herbs, *1*: 209
Fresh pasta, *1*: 339
  classification criteria, *1*: 340
  cooking quality, *1*: 333
  kneading and sheeting, *1*: 345
  packaging, *1*: 350
  pasteurization, *1*: 349
  quality characteristics, *1*: 331, 335, 367
  raw materials, *1*: 343
  shelf-life, *1*: 352
  stuffed pasta, *1*: 341
  water activity, *1*: 196
Friable, *2*: 480
Fromage frais, *1*: 652
Frost, *2*: 863, 865, 867, 871–873, 875
Frozen
  products, *2*: 474
  yogurt, *2*: 474
Frozen avocado halves, *2*: 1008
  cold chain, *2*: 1008
  IQF belt freezer, *2*: 1008
Frozen avocado paste and guacamole, *2*: 1003–1009
  avocado paste, *2*: 1005
  blast freezer, *2*: 1007
  cryogenic freezer, *2*: 1007
  freezing, *2*: 1006
  frozen storage, *2*: 1007
  guacamole, *2*: 1006
  liquid nitrogen, *2*: 1007
  packaging, *2*: 1006
  quality standard, *2*: 1007
  sorting and grading, *2*: 1004
Frozen avocados, *2*: 997–1011
  biology, *2*: 999
  climateric, *2*: 1000
  Hass avocado, *2*: 1001
  nutritional value, *2*: 1003
  oil, *2*: 999
Frozen bakery products, except bread, *1*: 103
Frozen chicken parts, *1*: 12
Frozen cream, *2*: 600
Frozen desserts, *2*: 593
  classification, *2*: 595
  composition, *2*: 597
  federal standards, *2*: 596
  formulation, *2*: 597
  mix calculation, *2*: 606
  trade classification, *2*: 596
  trends in market, *2*: 596

Frozen fish fillet blocks, *2*: 826–829
  defects, *2*: 826
    belly flaps, *2*: 827
    blood spots, *2*: 827
    bones, *2*: 828
    bruises, *2*: 827
    dehydration, *2*: 826
    discoloration, *2*: 827
    fins, *2*: 828
    foreign material, *2*: 828
    improper fill, *2*: 827
    membrane, *2*: 828
    parasites, *2*: 828
    scales, *2*: 828
    skin, *2*: 827
    texture, *2*: 828
    underweight, *2*: 826
    viscera, *2*: 827
Frozen fish, whiting, *2*: 822–823
  grading, *2*: 822
    bruises, *2*: 823
    color, *2*: 823
    dehydration, *2*: 823
    evisceration, *2*: 823
    flavor and odor, *2*: 823
    heading, *2*: 823
    minimum Size, *2*: 823
    scaling, *2*: 823
    texture, *2*: 823
Frozen foods, *2*: 857, 859–860, 862–863, 868–870, 873, 876, 880–881
Frozen fried fish portions, *2*: 839–840
  composition, *2*: 839
  examination, *2*: 840
Frozen fried fish sticks, *2*: 837–840
  composition, *2*: 836
  examination, *2*: 836
Frozen fruits, fruit juices, and vegetables, *1*: 97
Frozen halibut steaks, *2*: 823–824
  defects, *2*: 824
    dehydration, *2*: 824
    discoloration, *2*: 824
    honeycombing, *2*: 824
    non-uniformity, *2*: 824
    texture, *2*: 824
    workmanship, *2*: 824
  styles, *2*: 823
    random weight, *2*: 823
    uniform weight, *2*: 823
Frozen ham, *2*: 236
Frozen headless dressed whiting, *2*: 822–823
Frozen minced fish blocks, *2*: 829–832
  color classifications, *2*: 829
  defects, *2*: 829
    angles, *2*: 830
    blemishes, *2*: 831
    bones, *2*: 831
    dehydration, *2*: 830
    discoloration, *2*: 829
    flavor and odor, *2*: 831
    improper Fill, *2*: 830
    texture, *2*: 829
    uniformity of size, *2*: 830
    uniformity of weight, *2*: 830
  texture, *2*: 829
  types, *2*: 829
Frozen raw breaded fish portions, *2*: 835–837
  composition, *2*: 836
  examination, *2*: 836
  styles, *2*: 836
Frozen raw breaded fish sticks, *2*: 833–835
  composition, *2*: 834
  definitions, *2*: 834
  examination, *2*: 834
Frozen raw breaded scallops and frozen fried scallops, *2*: 847–849
  definitions, *2*: 848
  description, *2*: 847
  styles, *2*: 848
  texture, *2*: 849
  types, *2*: 848
  workmanship defects, *2*: 848
Frozen raw breaded shrimp, *2*: 843–846
  styles, *2*: 843
Frozen raw fish portions, *2*: 832–833
  defects, *2*: 832
    blemishes, *2*: 832
    bones, *2*: 833
    dehydration, *2*: 832
    texture defects, *2*: 833
    uniformity of shape, *2*: 832
    uniformity of size, *2*: 832
  styles, *2*: 832
  types, *2*: 832

Frozen raw scallops, *2*: 846–847
definitions, *2*: 847
styles, *2*: 846–847
types, *2*: 847
Frozen salmon steaks, *2*: 824–826
defects, *2*: 826
discoloration, *2*: 826
honeycombing, *2*: 826
non-uniformity, *2*: 826
texture, *2*: 826
dimensions, *2*: 826
species, *2*: 824
styles, *2*: 825
random weight, *2*: 825
random weight combination, *2*: 825
uniform weight, *2*: 825
Frozen specialities, not elsewhere classified, *1*: 97
Frozen storage and retailing, *2*: 924
Fructose, *1*: 468, 689, *2*: 639
Fruit, *2*: 404, 406
banana, *2*: 404–406
date, *2*: 405
diet, *2*: 57–58
flavonoids, *2*: 58
grape, *2*: 404
mango, *2*: 405–407
orange, *2*: 404–406
raspberry, *2*: 404, 406
strawberry, *2*: 404, 406–407
Fruit juice processing, *1*: 855
Fruit juices, *1*: 855
packaging, *1*: 857–859
storage, *1*: 861
FUM1 gene, *1*: 476
Fumarase, *1*: 476, 489
Fumarate reductase, *1*: 476
Functional food(s), *1*: 904–906, 913–928, 1000–1004, 1007, 1023, *2*: 60, 120, 154, 446
biological effects, *1*: 914–922
consumers' focus on prevention of illness, *1*: 947
definition, *1*: 906
dietary guidance, *1*: 929–930
effects on major chronic diseases, *1*: 913–914
examples, *1*: 906
fish oil, *2*: 116, 120, 128
DHA, *2*: 116, 120, 128
EPA, *2*: 116, 120, 128
inflammation, *2*: 120
history of use, *1*: 945
meat-based
animal production practises, *1*: 995, 1003
meat transformation systems, *1*: 999
probiotic, *2*: 120, 124, 127
public education, *1*: 929–930
regulation of, *1*: 927–929
safety issues, *1*: 924–925
safety and toxicology aspects related to functional, *1*: 945
the global market, *1*: 942, 953
the rising cost of health-care related to chronic disease, *1*: 943
the role of food in prevention strategies, *1*: 945
Functional foods and herbs, *2*: 141
Broccolis, *2*: 141, 143
calcium, *2*: 142
cocoa and chocolate, *2*: 142
coffees (*Coffea arabica*), *2*: 144
curcumin (diferuloylmethane), *2*: 143
fish, fish oil, and olive oil, *2*: 141
fruit and vegetable fibers, *2*: 143
garlic, *2*: 143–144
grapes and wine, *2*: 144
propolis, *2*: 143
soy foods, *2*: 142
teas (Camellia sinensis), *2*: 144
tomato, *2*: 138, 141
vitamin A, *2*: 138
Functional foods ingredients, *1*: 904
Functional foods, nutraceuticals, *1*: 958
Europe, *1*: 941
Japan, *1*: 941
United States, *1*: 941
Functional ingredients, *1*: 959, 961
Functional properties, *2*: 40, 42, 44, 46, 48, 50
Functional properties of milk, *1*: 971
enhancement by culturing, *1*: 984
Functional role of calcium and other minerals, *1*: 982
Functionality, *1*: 311
Fungi, *1*: 33
Furaneol, *1*: 798, 836, 855

Furanones, *1*: 798
Furans, *1*: 850
Furfural, *1*: 517, 828
Fusel alcohols, *1*: 181–182, 471, 475

α-Glucosidase, *1*: 188, 468, 481
β-Glucan, *1*: 445, 449–451
β-Glucosidase, *1*: 188
Galactosaemia, *2*: 582
Galactose, *2*: 391
Gallstones, *2*: 480
Gangliosides, 2: 686
Garlic, *1*: 79, 121–122, 127
Gas chromatography, *1*: 210, 392–400, 845
Gas chromatography olfactometry/
  aroma extract dilution analysis, *2*: 731
Gas chromatography–mass spectrometry,
    1: 141, 210, 759
Gas laws of Henry, Boyle, and Charles, *1*: 422
Gas-solid fluidized-bed, *1*: 199
Gastrointestinal, *2*: 163, 450
  allergy, *2*: 479
  disorders, *2*: 450, 454
GC. *See* Gas chromatography
GC-MS. *See* Gas chromatography–mass
    spectrometry
GCO/AEDA. *See* Gas chromatography
    olfactometry/aroma extract dilution
    analysis
GCO-H. *See* Static headspace-olfactometry
GDP1 gene, *1*: 471
Gelatin, *2*: 605
Gelatinization, *1*: 291, 308
Gelation, *2*: 387
Gelato, *2*: 595
Gene
  coding, *2*: 471
  frequency, *2*: 470
Generally recognized as safe (GRAS),
    1: 35, *2*: 202
Genetic, *2*: 452
  polymorphism, *2*: 464, 472
  rearrangement, *2*: 472
  selection, *2*: 452
  typing, *2*: 472
Genetic engineering, *1*: 171–174
Genetically modified organism (GMO),
    1: 194, 301
Genoise cake, *1*: 318
Genotype, *2*: 471
Geosmin, *1*: 187
Geotrichum candidum, *2*: 412, 425
Geotrichum penicilliatum, *1*: 185
Geranial, *1*: 852
Geranic acid, *1*: 187
Geranyl acetate, *1*: 853
Ghee, *2*: 478
Ginger, *1*: 926
Gingko biloba, *1*: 926
Ginseng, *2*: 171–172, 174–182, 184–193
Ginsinoside(s), *2*: 176–179, 181, 184,
    186–193
Gjetost, *2*: 478
Glass transition temperature, *1*: 406, 872
Glazes, *2*: 887
Glucose, *1*: 467–469, 689, 850, *2*: 465, 639.
    See also Carbohydrates
Glucosinolates, *1*: 913
Glutamic acid, *1*: 183, 994, *2*: 887
Glutathione, *1*: 886, 977
Glutathione peroxidase, *2*: 469
Gluten, *1*: 100, 263, 265, 284
  replacers, *1*: 531
  vital wheat gluten, *1*: 294–295, 299, 1000
Gluten-free
  bread(s), *1*: 1021–1023,
  cereals, *1*: 1021
  diets, *1*: 1019, 1021
  foods, *1*: 1024
Gluten-free products, *1*: 376, 531
Gluten proteins
  gliadin, *1*: 265, 1018, 1021
  glutenin, *1*: 265
  prolamin, *1*: 1081, 1021
Glyceride, *2*: 472
Glycerin, *2*: 639
Glycerol, *1*: 710, *2*: 458, 472
  ethers, *2*: 458
Glycerol-3-phosphate dehydrogenase, *1*: 471
Glycine betaine, *2*: 905
Glycine, *2*: 936
Glycolysis, *1*: 471, *2*: 271, 284, 877
Glycolysis and related reactions, *2*: 719
GM, *1*: 284
GMPR, *2*: 950, 954
GMPs. *See* Good manufacturing procedures

Goat, *1*: 569, 739, *2*: 380–381, 383–387, 389–391, 393–395, 477
keepers, *2*: 454
pedigreed, *2*: 454
population, *2*: 451
Goat breeders, *2*: 454
Goat breeds, *2*: 459
alpine, *2*: 452, 467–468, 480
Anglo-Nubian, *2*: 452, 460, 468
Canaria, *2*: 452
Damascus, *2*: 452
French-Alpine, *2*: 468
Garganica, *2*: 471
Indian Jamunapari, *2*: 452
indigenous, *2*: 460
African Dwarf, *2*: 460
Red Sokoto, *2*: 460
La Mancha, *2*: 460
Malaguena, *2*: 452, 471
Maltese, *2*: 452, 471
Murciana-Granadina, *2*: 452, 471
Nubian, *2*: 460, 480
Oberhasli, *2*: 452, 460
Poitevine, *2*: 471
Saanen, *2*: 452, 460, 470–471
Swiss, *2*: 460
Toggenburg, *2*: 452, 460
Goat cheese, *2*: 454
Goat milk, *2*: 450–451, 454, 456
anemia, *2*: 481
cheese, *2*: 454
artisanal, *2*: 454
Chabicou, *2*: 476
Chabis, *2*: 476
Crottin du Chavignol, *2*: 476
Les Pyramides, *2*: 476
Sainte Maure, *2*: 476
cheesemaking, *2*: 456
chemical composition, *2*: 486
fluid, *2*: 474
production, *2*: 450–451
economics, *2*: 456
products, *2*: 478
cosmetic, *2*: 478
evaporated, *2*: 474
fermented, *2*: 477
frozen, *2*: 454
powdered, *2*: 474
uniqueness, *2*: 458
yogurt, *2*: 477
Goaty flavor, *2*: 463
Good manufacturing practices (GMP), *1*: 224, 284, *2*: 343, 949
clean room processing environment, *2*: 343
compliance with public health regulations, *2*: 344–345
employee training, *2*: 343
written standard operating procedures, *2*: 343
Good manufacturing procedures, *1*: 217
Gorgonzola, *2*: 501, 509
Gouda, *1*: 188, *2*: 496, 498, 501–502
Grade A milk, *2*: 389
Grade A pasteurized milk ordinance (PMO), *2*: 332–333
Grade(s), *2*: 1055–1056
Grain mill products, *1*: 98–102
Granulated sugar, *1*: 314
Grape juice, *1*: 848, 855
Grapefruit juice, *1*: 848–853
Grapes, *1*: 855
Greenland halibut, *2*: 932
Grit, *2*: 1057, 1061, 1062
Growth parameters, *2*: 481
Gruyere, *1*: 598
Guaco, *1*: 166
Guaiacol, *2*: 726
Guar gum, *2*: 605
Guernsey, *2*: 381
Guide to Nutritional Labeling and Education Act (NLEA) requirements, *1*: 255
Guinea pig, *2*: 393
Gums, *1*: 60, 599–966, *2*: 404–405

1-Hexanol, *1*: 860
2,4-Heptadienal, *2*: 940
3-Hexen-1-ol, *1*: 856
3-Hexenal, *1*: 856
3-Hydroxy-2-butanone, *2*: 719
4-Hydroxydecanoic acid, *1*: 197
5-Hydroxymethylfurfural, *1*: 458
4-Hydroxy-2,5-dimethyl-3(2H)-furanone, *1*: 861
α-Hydroxy acids, *2*: 725
α-Hydroxyglutarate, *1*: 476

H_2S, *1*: 480, 717
HACCP (Hazard Analysis Critical Control Points), *1*: 10, 224–241, *2*: 343, 909, 946, 948, 950
  frozen shellfish, *2*: 909
Haddock, *2*: 932
HADH. *See* Hydroxyl acid dehydrogenase
Ham. *See* Cooked ham, Dry-cured ham
Hama-natto, *1*: 73
Hams, *1*: 63, 65
Hand lotion, *2*: 478
Hand-cut noodles, *1*: 544
Hand-swung noodle, *1*: 544
Hand-washing facilities, *1*: 230
Hansenula anomala, *1*: 185
Hansenula mrakii, *1*: 185
Haplotype, *2*: 471
Hard drinks, *1*: 420
Harusame, *1*: 541, 559, 561
Havarti, *2*: 502
Hay, *2*: 456
Hayamugi, *1*: 543
HDL cholesterol, *1*: 918, 923
HDPE. *See* High-density polyethylene
Headspace vapor analysis, *1*: 140
Healing, *1*: 579, 624
Health, *2*: 463
  food store, *2*: 457
Health Canada, *1*: 283
Health claims, *1*: 283, *2*: 70
  qualified health claims, *1*: 924
    antioxidant vitamins and cancer, *1*: 924
    folic acid and neural tube birth defects, *1*: 924
    nuts and heart disease, *1*: 924
    omega-3 fatty acids and coronary heart disease, *1*: 924
    phosphatidylserine and cognitive dysfunction and dementia, *1*: 924
    selenium and cancer, *1*: 924
Health concerns, *1*: 280
Health promoting ingredients, *1*: 968
Heat, *2*: 218, 220, 473
  coagulation, *2*: 474
  coefficients, *2*: 220
    cheese, *2*: 218
    meats, *2*: 220
  conductivity, *2*: 219–220
  desiccated, *2*: 478
  exchangers, *2*: 479
  heat transfer, *2*: 222
  penetration, *2*: 228
  stability, *2*: 473
Heat application, *1*: 24
Heat blanching, *1*: 234
Heat capacity, *2*: 370
Heat concerns, *1*: 280
Heat exchangers, *1*: 25–28
Heat removal (cold preservation), *1*: 26
Heat shock, ice cream, *2*: 628–629
Heat stability, *2*: 372
Heat transfer, *1*: 322
  conduction, *1*: 322
  convection, *1*: 322
  radiation, *1*: 322
Heavy metals, *1*: 216
Hemoglobin, *2*: 470, 479
  iron, *2*: 481
  regeneration efficiency, *2*: 481
Herbicide, *2*: 474
Herbs, *1*: 206, 208–209, *2*: 176, 178
  freeze-drying, *1*: 209
  in the food industry, *1*: 217–219
Herbs and spices
  quality aspects, *1*: 215–217
Herds, *2*: 459
Heritable, *2*: 471
Herring, *1*: 111
Heterocyclic amines, *1*: 1006–1007
Hexanal, *1*: 849, 856
Hexanoic acid, *2*: 726
Hexanol, *1*: 186
Hexose transporters, *1*: 468
Hexyl acetate, *1*: 851–852
High altitude adjustment, *1*: 316–317
High flavor impact, *1*: 121
High fructose corn syrup (HFCS), *1*: 430
  sensory active compounds, *1*: 430
High pressure processing (HPP), *1*: 40
High ratio sugar cake, *1*: 309
High vacuum isolation/aroma extract dilution analysis, *1*: 732
High-density polyethylene, *1*: 859
Higher alcohols, *1*: 181–182, 458, 471–473, 477
  aliphatic, *1*: 471

aromatic, *1*: 471
biosynthesis, *1*: 471
High-voltage arc discharge, *1*: 41
Hinterland, *2*: 454
Hiramen, *1*: 544
Hispanic cheeses, *1*: 648
Histamine. *See* Amines
HMF. *See* 5-Hydroxymethylfurfural
Ho fen, *1*: 540
Hokkien-type noodles, *1*: 552
Holstein, *2*: 381
Homeostasis, *2*: 48
Homerus americanus, *2*: 896
Homofuraneol, *2*: 732
Homogenization, *1*: 575, 609, 621. *See also* Emulsion
homogenizers, types of, *1*: 729–730
lipolysis, effect on cream, *1*: 732–733
two-stage, *1*: 729, 731–732
Homogenized, *2*: 384
milk, *2*: 461
Homology, *2*: 470
Homozygous, *2*: 472
Honey, *1*: 310
Hormone, *2*: 47, 49
Horse, *2*: 380
Human, *2*: 385, 392
milk, *2*: 467
nutrition, *2*: 458
Hydration, *2*: 472
degree of, *2*: 473
Hydrocolloids and nonfish proteins, *2*: 918
Hydrocolloids, *1*: 604, 613, 1027, *2*: 605, 637–638
Hydrodistillation, *1*: 149, 153–155
Hydrogen sulfide, *1*: 480
Hydrolysates, *2*: 698, 705
Hydrolysis, *2*: 491, 493–496, 501, 503, 509
Hydroxyl acid dehydrogenase, *1*: 725
Hydroxymethylfurfural, *1*: 861
Hydroxypropylmethylcellulose (HPMC), *1*: 531, 1028
Hygiene, *2*: 859–860
Hypercholesterolaemia, *2*: 168
Hyperglycemia, *2*: 176–177
Hyperlipoproteinemia, *2*: 480
Hypersensitivity, *2*: 118–119
classification, *2*: 118
Hyperuricaemia, *2*: 582
Hypocholesterolemic, *2*: 480
Hypocholesterolemic peptides, *2*: 496–497
Hypoxanthine, *2*: 858, 907

2-Isobutyl-3-methoxypyrazine, *2*: 733
β-Ionone, *1*: 187
IAH1 gene, *1*: 475
Ice, *1*: 235, *2*: 857, 860–864, 873, 876, 881–883, 886, 887
Ice cream, *1*: 733–734, *2*: 594
formulation, *2*: 597
market trends, *2*: 596–597
nutritional value, *2*: 630, 632
premium, *2*: 596
role of components, *2*: 600
shelf life, *2*: 626
Ice-cream and frozen desserts, *1*: 93
Ice cream freezers, types, *2*: 622
continuous, *2*: 622–623
Ice cream novelties, *2*: 630
extruded novelties, *2*: 632–633
molded novelties, *2*: 630–631
IDH1 gene, *1*: 476
IDH2 gene, *1*: 476
ILV pathway, *1*: 477, 479
Imitation flavors, *1*: 139
Immobilized cell technology, *1*: 485
applications, *1*: 482
Immune, *2*: 41, 43–45, 49
Immunity, *2*: 157
Immunoglobulins, *1*: 978, *2*: 358, 384, 465
Immunological
cross-reaction, *2*: 479
Immunomodulator, *2*: 157
Immunomodulatory peptides, *2*: 495, 510
Inactivated, *2*: 468
Incentive, *2*: 457
payment, *2*: 457
Incidental, *2*: 457
sale, *2*: 457
Incubation, *2*: 477–478
Indirect dryer, *1*: 164
Indole, *2*: 905, 909
Industrial process, *2*: 58–59, 62
Infant formula,
soy-based, *2*: 481

Infant formulas
carbohydrates, *2*: 688
functional compounds, future, *2*: 706
intestinal microflora, *2*: 689
lipids, *2*: 679
long chain polyunsaturated fatty acids (LCPUFA), *2*: 680
oligosaccharides, *2*: 690, 692
prebiotic factor, *2*: 690, 692
preformed dietary LCPUFA as components of biological membranes, *2*: 680
recommendations for the supplementation of infant formulas, *2*: 685, 692
Infant nutrition, *1*: 969, *2*: 579
Infection, *2*: 155
Inflammation, *2*: 155
Inflammatory bowel disease, *2*: 161, 166
Infrared spectroscopy, *1*: 141
Infusion, *1*: 452, 789
Ingredients, *1*: 262–265, 272, 274, 609, 611, 614
fat, *1*: 263, 266–267
flour, *1*: 262–265, 267
gluten, *1*: 263–265, 267, 274
salt, *1*: 266, 272
sugar, *1*: 264, 266–267
water, *1*: 262, 265–269, 271
Inhibitors, *1*: 587
Inoculation, *2*: 477
Inorganic salts, *1*: 930
Inosine, *2*: 858
In-process sampling, *1*: 697
Insect(s), *2*: 950–951, 953–955, 957, 1056–1057, 1059
Insecticide, *2*: 474
Insertions, *2*: 472
Instant noodles, *1*: 550, 552, 562
Institute of Medicine (IOM)
Food and Nutrition Board, *1*: 906, *2*: 208, 211–212
Intensity, *2*: 464
International Life Science Institute, *1*: 906
International Society of Beverage Technologists (ISBT), *1*: 433
analytical procedure for bottler's carbon dioxide, *1*: 433
quality guidelines for bottler's carbon dioxide, *1*: 433
Intestinal resection, *2*: 480
Intolerance, *2*: 392
Inverse relationship, *2*: 460, 468, 473
Inversions, *2*: 472
Invertase, *1*: 188
Iodine, *2*: 458
values, *2*: 472
Iodine index, *2*: 235, 256
Ionizable group, *2*: 465
Irish whiskey, *1*: 517
distillation, *1*: 517
new spirits, *1*: 517
strong feints, *1*: 517
strong low wine, *1*: 517
weak feints, *1*: 517
weak low wine, *1*: 517
Iron, *1*: 993, 998, 1007, 1009, *2*: 390, 458
Irradiation, *1*: 126, 857–858
Irritable bowel syndrome, *2*: 163
Isoamyl acetate, *1*: 183, 185–186, 196, 475, 820
Isoamyl alcohol, *1*: 182, 196
Isoamyl alcohol acetyltransferase, *1*: 475
Isobutanol, *1*: 185–186, 472
Isobutyl acetate, *1*: 185–186, 56
Isobutyl alcohol, *1*: 182
Iso-butyric acids, *1*: 182
Isoelectric point, *2*: 877
Isoflavones, *1*: 911, 915, 999, 1004, 1006, *2*: 57, 394
Isolated soy protein (ISP), *1*: 923
Isolation, *2*: 465
Isoleucine, *1*: 975
Isopenthyl acetate, *1*: 185
Isothermal heating system, *1*: 393
Iso-valeric acids, *1*: 182
Isracidin, *2*: 490, 504–505, 509
Italico, *2*: 501

Japanese whisky, *1*: 518
Jarlsberg, *2*: 499–501, 503
Jersey, *2*: 381
Juice concentration, *1*: 858
Juice extraction, *1*: 856
Juice post-extraction treatments, *1*: 856
Juice treatments
thermal and non-thermal, *1*: 856
Juices, *2*: 636, 869

5-Ketododecanoic acid, *1*: 196
α-KA. *See* α-Keto acids
α-Keto-β-methylvaleric acid, *1*: 473
α-Keto acids, *1*: 472–473
α-Ketoglutarate dehydrogenase, *1*: 475
α-Ketoglutarate, *1*: 476
α-Ketoisocapric acid, *1*: 473
α-Ketoisovaleric acid, *1*: 473
β-Ketoacids, *2*: 720, 722
Kappacin, *2*: 500, 504, 506, 509
Kashk, *2*: 478
Kava kava products, *1*: 926
Kefir manufacturing, *1*: 62
Kefir, *2*: 100–101, 412, 423, 432, 478, 661
  grains, *2*: 478
  kefir grain, *2*: 423–424
  microflora, *2*: 423
  processing, *2*: 443–444
Ketones, *1*: 181, 209–210
Kettles, *1*: 26
Kettling, *1*: 297
Khoa, *2*: 478
Kidding, *2*: 460
Kidneys, *2*: 210
Kimchi, *1*: 79
Kirsimen, *1*: 544
Klebsiella pneumoniae, *2*: 495
Kluyveromyces, *1*: 181
Kluyveromyces lactis, *1*: 187
Kluyveromyces marxianus, *1*: 185
k_m, *2*: 465
Kneading, *2*: 478
Knife-cut noodles, *1*: 544
Konjac (Konjak) flour, *1*: 561
Konjac (Konjak) yam, *1*: 561
Koumiss, *2*: 412, 423
  microflora, *2*: 423
Krausen, *1*: 478
Krebs cycle, *1*: 476
Kuan mien, *1*: 544
Kumys, *2*: 102
K-value, *2*: 909

α-Lactalbumin, *1*: 977, *2*: 357, 464
β-Lactoglobulin, *1*: 977, *2*: 357, 464
δ-Lactones, *1*: 187
γ-Lactones, *1*: 720
L. reuteri, *2*: 446
Labor cost, *2*: 456
LACF, *2*: 948
Lactaid, *2*: 391
Lactalbumin, *2*: 491–493, 500, 506
Lactase, *1*: 188, 284, *2*: 391
Lactate, *1*: 715
Lactation, *2*: 456
Lactenin, *2*: 504–505
Lactic acid, *1*: 181, 604, 636, *2*: 257, 266, 268, 271, 284, 315, 390, 478
Lactic acid bacteria fermentation, *1*: 718
Lactic acid bacteria (LAB), *1*: 362, 1023, *2*: 88–89, 97–99, 491, 503, 510, 718
  Lactobacillus sanfranciscensis, *1*: 363
  non starter, *2*: 89
  starters, *1*: 362
  viable, active, *2*: 99
Lactic acid bacterium function, *2*: 446–447
Lactitol, *2*: 639
Lactobacilli, *1*: 157
Lactobacillus
  acidophilus, *2*: 478
  bulgaricus, *2*: 477
Lactobacillus acidophilus, *2*: 96, 98, 446–447, 500, 502, 652, 664, 667–668
Lactobacillus bulgaricus, *2*: 96, 98–99, 647, 650, 653, 657, 662, 667
Lactobacillus casei, *1*: 478, *2*: 501
Lactobacillus delbrueckii subsp. *bulgaricus*, *2*: 501
Lactobacillus helveticus, *2*: 494, 497–499, 501, 506, 509
Lactococcus casei, *1*: 478
Lactococcus lactis, *1*: 571, *2*: 446
Lactococcus lactis, cremoris, *2*: 99
Lactococcus lactis subsp. *cremoris*, *2*: 501, 503
Lactococcus lactis subsp. *lactis*, *2*: 502–503
Lactoferricin, *2*: 490, 492–493, 500, 504, 507–509
Lactoferrin, *1*: 959, *2*: 358, 465, 490, 492–493, 503–504, 507–509
Lactoglobulin, *2*: 491–493, 496, 500, 502, 506–507, 509
Lactokinins, *2*: 497, 500
Lactones, *1*: 187
Lactoperoxidase, *2*: 360, 388
Lactorphins, *2*: 491, 493, 500

Lactose, *2*: 362, 381, 385, 390–391, 458–459, 466, 639
  crystals, *2*: 478
Lactose (also lactate), *1*: 588, 597, 604, 612, 654, 714–715, 735, 982
Lactose intolerance, *1*: 1020, *2*: 166, 581, 669
Lactose intolerance, alleviation, *2*: 105–106
La Mancha, *2*: 382
Lamiaceae, *1*: 208
Large intestines, *2*: 478
Latin American white cheese, *1*: 648
  manufacture, *1*: 638
Laurel, *1*: 209
Lauric acid, *1*: 718
Lautering, *1*: 452
Layout, *2*: 870–871
Lb. bulgaricus, *2*: 438
L-carvone, *1*: 210
L-cysteine, *1*: 861
LDL cholesterol, *1*: 922–923
LDPE. *See* Low-density polyethylene
Leaching, *1*: 150
Leafy greens, frozen, *2*: 990
  styles, *2*: 990
    chopped, *2*: 990
    leaf, *2*: 990
    pureed, *2*: 990
  types, *2*: 990
    beet, *2*: 990
    collards, *2*: 990
    dandelion, *2*: 990
    endive, *2*: 990
    kale, *2*: 990
    mustard, *2*: 990
    spinach, *2*: 990
    Swiss chard, *2*: 990
    turnip, *2*: 990
Leavening agents, *1*: 289, 311
  air, *1*: 216
  baking powder, *1*: 289, 312
  baking soda, *1*: 289, 312
  sodium bicarbonate, *1*: 289, 312
  steam, *1*: 311–312
Lecithin, *1*: 313, 730
Legislative guidelines, *1*: 135
Length, *2*: 1052
Lentinus edodes, *1*: 183
Leu. citrovorum, *2*: 437, 439, 444
LEU4 gene, *1*: 475
Leucine, *1*: 473, 477, 975
Leuconostoc, *1*: 181, 619, *2*: 101, 103
Leuconostoc mesenteroides, *2*: 502
Lg-ATF1 gene, *1*: 475
Light activated off flavor, *2*: 655, 660
Light ice cream, *2*: 595
Lighting, *2*: 872
Lima beans, frozen, *2*: 987
  lumin, *2*: 154
  types, *2*: 987
    fresh-seed, *2*: 987
    thick-seeded, *2*: 987
    thin-seeded, *2*: 987
Limonene, *1*: 187, 210, 392, 394, 400–408, 411, 413, 852–853, 857–861
Limonin, *1*: 853
Linalool, *1*: 210, 852–853, 857–858
Linalool oxide, *1*: 857–858
Linalyl acetate, *1*: 210
Line extension, *1*: 133
Linoleic acid, *2*: 383
Lipase (also lipolysis), *1*: 571, 573
Lipase, *2*: 383, 387–388, 392, 463, 468
  activity, *2*: 468
  lipoprotein, *2*: 468
Lipase, milk, *2*: 648–649, 652
Lipases, *2*: 239–240, 245–246, 256, 268, 272, 283
Lipid autoxidation, *2*: 858
Lipid hydrolases, *1*: 854
Lipid oxidation, *1*: 858, 997–998, 1005–1009, *2*: 240, 272–273, 717, 925
Lipids, *1*: 445, 458, 461, 473–474, 476, 533–534, 549, 570, 726–727, 745, 752, 982–984, 1001–1002, 1004, *2*: 239–241, 245–246, 256, 262, 272, 273, 283–285, 289–290, 305, 458, 461
  bound, *2*: 461
    glyco, *2*: 461
    neutral, *2*: 461
    phospho, *2*: 461
    polar, *2*: 461
  cholesterol, *1*: 990–993, 997, 999–1001, 1003, 1006
  fatty acids
    conjugated linoleic acid (CLA), *1*: 991, 993, 997

monounsaturated (MUFA), *1*: 991
polyunsaturated (PUFA), *1*: 991, 997, 1003
saturated (SFA), *1*: 991
trans, *1*: 991
free, *2*: 461
diglycerides, *2*: 461
monoglycerides, *2*: 461
triglycerides, *2*: 461
goat milk, *2*: 461
hydrolysis, *2*: 880, 884
meat, *1*: 990–991
oxidation, *1*: 997–999, 1005–1009, *2*: 882, 884, 886–888
protected, *2*: 459
Lipolysis, *1*: 726, 731–733, *2*: 239–240, 245–246, 262, 272, 283–284, 322, 383, 457, 463
spontaneous, *2*: 463, 468
Lipolysis and related reactions, *2*: 719
Lipoxygenase, *1*: 854, 856, 885
Liquid fermentation, *1*: 196–197
Liquid sugar, *2*: 602
Liquid(s), *1*: 290, 322, 406
in cakes, *1*: 310–311
Listeria, *2*: 457
Listeria innocua, *2*: 509
Listeria monocytogenes, *2*: 504
Live microbial food supplements, *1*: 920
Liver necrosis, *2*: 469
Lobster, *1*: 284, *2*: 882–883
American lobster, *2*: 897
blueing, *2*: 905
freezing and packaging requirements, *2*: 903–904
mushiness, *2*: 906
rock/spiny lobster, *2*: 896
Locality, *2*: 459, 476
Locust bean gum, *2*: 605
Longxu (dragon whiskers), *1*: 543
Lot, *1*: 225
Low flavor impact, *1*: 121
Low-density polyethylene, *1*: 859
Lutein, *2*: 61
Lycopene, *1*: 916, *2*: 1111–1112, 1115
heating, *2*: 59–60
plasma, *2*: 60–62
supplementation, *2*: 62
Lymphocytes, *2*: 389, 495
Lyophilization, *1*: 167
Lysine, *1*: 353
Lysozyme, *2*: 388, 467

1-Menthol, *1*: 187
2-Methyl butanol, *1*: 471
2-Methyl propanal, *2*: 939
2-Methyl-1,4-naphthoquinone, *2*: 49
2-Methyl-3-phytyl-1,4-naphthoquinone, *2*: 49
2-Methyl-1-propanol, *1*: 186
2-Methyl-3-furanthiol, *1*: 859, 861
2-Methylbutanoate, *1*: 856
2-Methylbutyl acetate, *1*: 781
2-Methyl-ethyl-butyrate, *1*: 185
3-(Methylthio)-propanal, *2*: 938
3-Methylbutanal, *1*: 849
4-Mercapto-4-methylpentan-2-one, *1*: 853
4-Methyl octanoic acid, *2*: 731
4-Methyoctanoic acid, *2*: 464
5-Methylfurfural, *1*: 857
6-Methyl-hexadecanoate, *2*: 463
β-Myrcene, *1*: 852–853, 861
Macaroni, spaghetti, vermicelli, and noodles, *1*: 113
Mace oil, *1*: 211
Maceration, *1*: 211. *See also* Extraction with hot fat
Machine, *2*: 869
Mackerel, *2*: 857, 885–886
fillets, *2*: 887
Macrobrachium rosenbergii, *2*: 895
Mad cow disease, *1*: 133
Magnesium, *1*: 921, 959, *2*: 458
Maillard, *1*: 451–452, 457
Maillard browning, *1*: 301, 310, 318, 410
Maillard reaction, *1*: 9, 126, 287, 310, 352–353, 363, 366, *2*: 245–246, 286–289, 296
dried pasta, *1*: 329
fresh pasta, *1*: 329
Major tropical fruits and products: banana, mango, and pineapple, *1*: 815–844
MAL gene, *1*: 469
Malabsorption, *2*: 480
Malate, *1*: 476
Malic acid, *1*: 854
Malnutrition, *2*: 451
Malt, *1*: 108–109, 133, 174, 295, 297, 301

Malt beverages, *1*: 108
Malt extract, *1*: 108, 295, 301
Maltase, *1*: 188, 449, 451, 469
Malting, *1*: 444, 450–451, 458
  germination, *1*: 508
  kilning, *1*: 458–459
  steeping, *1*: 449, 508
Maltitol, *2*: 639
Maltodextrins, *2*: 603
Maltol, *1*: 430
Maltose, *1*: 301, 356, 363, 373, 450
  metabolism, *1*: 451
  transporter, *1*: 469
Maltotriose, *1*: 450, 468, 470
  metabolism, *1*: 451
Mammary gland, *2*: 382
Management system, *2*: 456
Manchego, *2*: 499, 501
Mandarin juices, *1*: 857
Mango, *1*: 826–833
  antioxidant capacity, *1*: 821, 829
  classification, *1*: 826
    Indian, *1*: 826
    Indochinese, *1*: 826
  cultivation, *1*: 826
  flavor, *1*: 828
  harvest, *1*: 827
    climacteric, *1*: 827
    ethylene ripening, *1*: 827
  nutritional content, *1*: 828
  phenolics, *1*: 821, 836
  processed products and processing, *1*: 829–833
    aseptic-packed, *1*: 829–830
    chutneys, *1*: 829
    concentrate, *1*: 832, 851, 858–859
    infused dried, *1*: 832
    juice, *1*: 829–832
    leather, *1*: 832
    other mango products, *1*: 833
    puree, *1*: 829–830, 832
  storage, *1*: 827
    chilling injury, *1*: 827
  supply, *1*: 827
Manufactured ice, *1*: 113
Manufacturing, *2*: 473
  procedures, *2*: 476
  techniques, *2*: 476
  technology, *2*: 477
Manufacturing coding system, *1*: 254
Manufacturing of processed cheese
  blending, *1*: 600, 607
  cheese selection, *1*: 600
  cooking, *1*: 598, 607, 609
  cooling, *1*: 602, 609
  creaming, *1*: 607
  homogenization, *1*: 609
  packaging, *1*: 609–610
  pasteurization, *1*: 609
Margarine, *1*: 705, 707
  consumer margarine and spreads, *1*: 715, 721
    dairy spreads, *1*: 720
    low fat margarine, *1*: 720
    soft table margarine, *1*: 719–720
  crystallization, *1*: 710–711, 713, 715, 717, 719–720
    polymorphism, *1*: 709
  evaluation, *1*: 754, 768
  hippolyte mege mouries, *1*: 705
  industrial products, *1*: 715–716
    bakery margarine, *1*: 716
    cake and cream margarine, *1*: 717–718
    puff pastry margarine, *1*: 711, 717
    shortenings, *1*: 716
  ingredients, *1*: 707–709, 712–713, 720
    antioxidants, *1*: 708–709
    β-carotene, *1*: 708
    citric acid, *1*: 709
    color, *1*: 709
    emulsifiers, *1*: 708–709, 711
    fat phase and types, *1*: 707–709, 711, 713, 721–722
      beef tallow, *1*: 717
      hydrogenated, *1*: 708, 717–719, 721–722
      interesterified fat, *1*: 721
      lauric oils, *1*: 718
      palm, *1*: 717–719
      SFC profile, *1*: 707–708, 717–719, 721
    flavor, *1*: 709, 718, 722
    gelatine, *1*: 720
    preservatives, *1*: 709
    salt, *1*: 707, 709, 713
    water phase, *1*: 707, 709, 711–713, 721
  microbiological stability, *1*: 883

milk proteins, *1*: 709, 737, 744
pin rotor machine, *1*: 713, 715–717
processing and product trends, *1*: 722
processing methods, *1*: 721
drum chilling, *1*: 706
scraped surface heat exchanger, *1*: 706
processing values for residence time by margarine type, *1*: 714
resting tube, *1*: 713, 715
stabilizers, *1*: 720
trans fat, *1*: 719
Marjoram, *1*: 209
Market growth, *1*: 132, 950
Market maturity, *1*: 132
Marketer, *1*: 139–140, 145, 520
Marsupial, *2*: 385
Mashing, *1*: 447–449, 453, 455, 464
Mass spectra, *1*: 141
Mass spectrometry, *1*: 141, 210, 217
Mass transfer, *2*: 222
Mass transfer limitation, *1*: 485
Mastitis, *2*: 389
Maturation, *1*: 478–483, 485, 488–490
Maturation chambers, *1*: 870
Maturity, *1*: 872
Meat
composition, *1*: 994–997, 999–1000, 1004, 1006–1007
processing, *1*: 994, 999, 1002, 1007, 1009
Meat packaging plants, *1*: 89
Meat products, *1*: 63, 89–91, 990–1010
bacon, *2*: 306
composition, *1*: 994–997, 999
cooked. *See* Cooked ham
fermented sausages. *See* Sausages
formulation, *1*: 1000, 1003–1007
frankfurters, *2*: 308–312
ham. *See* Dry-cured ham
reformulation, *1*: 994, 999–1000
roast beef, *2*: 305–308
types
cooked ham, *1*: 1003, 1005
fermented sausages, *1*: 1003
frankfurter, *1*: 1003, 1005
ground beef, *1*: 1001, 1003
pork liver pâté, *1*: 1003
restructured beef, *1*: 1005–1006
Mechanical damage, *2*: 1053
Mechanical operations, *1*: 835, 882–883
cutting and/or slicing, *1*: 882–884
peeling, *1*: 882–883
Mechanically deboned poultry meat, *2*: 776–780
color, *2*: 776
composition, *2*: 776
functional properties, *2*: 777
microbiological properties, *2*: 777
nutritional properties, *2*: 776
process, *2*: 776
sausages manufacture, *2*: 778–779
gelling polysaccharides, *2*: 779
textural profile analysis, *2*: 779
surimi process, *2*: 778
Medical, *2*: 450
afflictions, *2*: 450
needs, *2*: 451
Mediterranean region, *2*: 450
Medium flavor impact, *1*: 121, 128
Mee, *1*: 540
Melanosis, *2*: 882, 905
Mellorine, *2*: 595
Menaquinone-7, *2*: 49
Menippe mercenaria, *2*: 897
Mental association, *1*: 144
Menthol, *1*: 187, 210
Meringue method, *1*: 313, 315
MET gene, *1*: 480
Meta-analysis, *2*: 190–191, 204
Metabolic, *2*: 161, 458
Metabolism, *2*: 44, 46, 49–50, 458, 877
Metal drum, *2*: 479
Metallic, *2*: 390
Methanethiol, *2*: 720, 722, 729
Methional, *1*: 861, 936
Methyl anthranilate, *1*: 855
Methyl ketones, *2*: 722, 731
Methyl octanoate, *1*: 857
Methyl-3-buthanol, *1*: 186
Methylfuraneol, *1*: 855
Mi fen, *1*: 540
Micellar, *2*: 472
characteristics, *2*: 474
hydration, *2*: 473
mineralization, *2*: 473
size, *2*: 472
Micelle, *2*: 383
Micelle structure, *2*: 473

Microbial fermentation, *1*: 193–194
Microbial inspection, *1*: 696
Microbiology, *2*: 864
Microfiltration, *1*: 200, 856
Microorganisms, *1*: 170–174, 179–189, 225, 233, 235, *2*: 218, 226, 857. *See also* Starter cultures
  Alternaria, *2*: 218
  Bacillus coagulans, *2*: 219
  Bacillus macerans, *2*: 219
  Bacillus polymyxa, *2*: 219
  Candida. *See* Yeast
  Clostridium. *See* Pathogens
  Clostridium botulinum, *2*: 219
  Coxiella burnetti, *2*: 226
  Debaryomices. *See* Yeast
  Enterobacteriaceae, 810, 905
  Escherichia coli. *See* Pathogens
  Fusarium, *2*: 218
  Kocuria, *2*: 266–268, 272
  lactic acid bacteria, *2*: 253, 266–270, 284, 288
  Lactobacillus, *2*: 260, 266–271, 273
  Lactococcus, *2*: 416, 424
  Listeria. *See* Pathogens
  Micrococcaceae, *2*: 256, 266, 272, 316
  Pediococcus, *2*: 260, 266, 268, 316
  Penicillium. *See* Mold
  Pseudomonas. *See* Pathogens
  Salmonellae. *See* Pathogens
  Staphylococcus, *2*: 266–267, 272, 288, 316
  Mycobacterium tuberculosis, *2*: 226
  Pseudomonas, *2*: 219
  Rhizoctonia, *2*: 218
  spoilage, *2*: 218, 220
  Staphylococcus aureus, *2*: 219
  thermal resistance, *2*: 218, 220
Microsatellites, *2*: 473
Microwave, *2*: 873, 876, 883
Microwave and radio frequency processing, *1*: 38–39
Microwave foods
  lack of crispness and browning, *1*: 409–410
Microwave heating, *1*: 393, 409–411
Microwave heating of flavor-formulated dough, *1*: 409–413
Mie, *1*: 540
Mien (Mian, mein), *1*: 540
Mikania glomerata, *1*: 166
Milk, *1*: 618, 653, 713–738, *2*: 472–473, 488–498, 501–502, 504, 509–510
  acidophilus, *2*: 456
  ash content, *2*: 349
  beverage, *2*: 474
  caprine, *2*: 463, 468–469
  chemical composition of, *2*: 349
  composition, *2*: 472
  constituents, *2*: 349, 461
  defatted, *2*: 478
  definition, *2*: 349
  different breeds, *1*: 727
  factors affecting composition, *2*: 349
  fat depression, *2*: 459
  flavor, *1*: 713–738
  flavor characterization, *1*: 724–737
  flavor production reactions, *1*: 714–721
  milk composition, *1*: 570, 574, 576, 583, 587–588
  minor constituents, *2*: 365
  physical structure, *2*: 349, 366
  price, *2*: 452
    feed price, *2*: 454
  production, *2*: 454–456
  products, *2*: 451
  protein, *2*: 464
  raw, *1*: 570–571, 575–576, 584, *2*: 457
  skimmed, *2*: 478
  specific flavors, *1*: 731–734
  specific off-flavors, *1*: 731–734
  supply, *2*: 450
  taste compounds, *1*: 734–737
Milk and milk products, flavors of, *2*: 716
Milk constituents, *2*: 349
  major constituents, *2*: 351
    biogenic activity, *2*: 105
    fat, *2*: 349
    lactose, *2*: 362
    minerals, *2*: 363
    proteins, *2*: 353
    water, *2*: 349
Milk constituents, functional properties of, *1*: 973–986
Milk enzymes, *2*: 360
  alkaline phosphatase, *2*: 361
  lipoprotein lipase, *2*: 361
  lysozyme, *2*: 361
  protease, *2*: 361

Milk fat, *1*: 979–981, *2*: 106, 349, 351
cholesterol, *2*: 351
concentrated source, *2*: 597
conjugated linoleic acids, *1*: 979, *2*: 106
constituents, *1*: 978, *2*: 350
crystals, *1*: 734–735, 738–739, 741, 744
fat globule membrane, *2*: 351–352
composition, *2*: 352
fatty acid profile, *1*: 979–980, *2*: 351
fatty acids, *1*: 979, 980
phospholipids, *1*: 981, *2*: 106, 351, 355
physiological effects, *1*: 973, 976, 979
Milk fat content, *2*: 337
low fat products, *2*: 337
skim milk products, *2*: 337
thermalized cream, *2*: 337
whole milk products, *2*: 337
Milk fat globule membrane (MFGM), *1*: 726–728, 730, 733–735, 738
Milk lipids, *1*: 726–727
Milk physical properties, *2*: 364
Milk physical structure, *1*: 973
Milk products, *1*: 713–738
flavor, *1*: 713–738
flavor characterization, *1*: 724–737
flavor production reactions, *1*: 714–721
specific flavors, *1*: 731–734
specific off-flavors, *1*: 731–734
taste compounds, *1*: 734–737
thermally-induced changes, *1*: 715
Milk proteins, *1*: 971, *2*: 105, 355
caseins, *1*: 974, *2*: 355–356
alcohol coagulation, *2*: 364
heat coagulation, *2*: 358
isoelectric precipitation, *2*: 100, 357
peptides, *1*: 974, *2*: 101, 360
physical and chemical properties, *2*: 359
polyvalent ion precipitation, *2*: 358
rennet coagulation, *2*: 358
functional attributes, *2*: 362
Milk, chemical composition of, *1*: 972
Milk, functional properties of, *1*: 973
Milk, mineral composition, *1*: 982, *2*: 364
Milk, nutritional characteristics, *1*: 972
Milking
year-round, *2*: 461
Mill run, *2*: 1057
Milling, *1*: 448–449
Mince, *2*: 877, 881, 885
Mineralizations, *2*: 473
Minerals, *1*: 921, 942, 998, *2*: 363, 456, 466
calcium, *2*: 468
chloride, *2*: 468
chlorine, *2*: 468
magnesium, *2*: 468
Mo, *2*: 469
partition in milk, *2*: 365
phosphorus, *2*: 468
potassium, *2*: 468
selenium, *2*: 469
sodium, *2*: 468
sulfur, *2*: 468
zinc, *2*: 469
Misbranding, *1*: 137
Miscellaneous food preparations and kindred, *1*: 111–115
Mislabeled products, *1*: 699
Miso production, *1*: 75
Misshapen, *2*: 1053
Mix calculations, *2*: 606
arithmetic method, *2*: 607
milk and cream formula, *2*: 610
serum point method, *2*: 610
Mix freezing, ice cream, *2*: 619
hardening, *2*: 624
overrun, *2*: 621
refrigeration, *2*: 620
types of ice cream freezers, *2*: 622
Mix processing, aging, *2*: 615
blending, *2*: 613
flavors, *2*: 617
homogenization, *2*: 616
pasteurization, *2*: 615
Mix proof sheet, *2*: 611
Mixers, *1*: 319
continuous mixers, *1*: 320
AMF mixer, *1*: 321
Baker Perkins homogenizer, *1*: 321
Oakes continuous automatic, *1*: 320
Oakes marshmallow, *1*: 320
vertical dough mixers, *1*: 319
planetary, *1*: 319
pressure bowl, *1*: 319

Mixing methods–shortened cakes, *1*: 315, 318
  conventional meringue, *1*: 315
  conventional sponge, *1*: 315
    pastry blend, *1*: 315
    single stage, *1*: 315
  creaming, *1*: 314–315
Mixing, *1*: 24, 236, 291, 296, 299, 313–316, 319–320
MMS, *1*: 857. *See also* S-Methylmethionine sulfonium
Model flavor-dough system, *1*: 392
Modeling, *1*: 272–274
  artificial neural network, *1*: 272
  CFD, *1*: 272
  empirical model, *1*: 272–273
  kinetic model, *1*: 272
  mapping technique, *1*: 273
  mathematical model, *1*: 272, 274
Modifications, *2*: 476
Modified atmospheric packaging (MAP), *1*: 28, *2*: 261–262
Modified atmospheric storage (MAS), *1*: 28
Modified gas atmosphere, *1*: 28
Mohair, *2*: 454
Moiety, *2*: 480
Moisture, *1*: 595, 597, 604, 616
Moisture content, *1*: 216
Moisture migration, *1*: 390, 409
Mold, *2*: 640–641, 644. *See also* Molds
Molding starch and trays, *1*: 698
Molds, *2*: 268–269, 273, 304, 477, 653, 658, 661, 664
Molybdenum, *2*: 458
Monoglycerides, *1*: 287, 657, 708, *2*: 606
Monosaccharides, *2*: 468
Monosaturated, *2*: 385
Monosodium glutamate, *1*: 123
Monoterpene alcohols, *1*: 852
Monounsaturated fatty acids, *1*: 720
Morchella, *1*: 183
Morels, *1*: 183
Mouthfeel, *1*: 287, 301
Mozzarella, *1*: 607–608, 734, *2*: 509
Muffins, *1*: 280, 284–287
  bread-type, *1*: 286
  cake-type, *1*: 285–286, 292
  English, *1*: 280
  evaluation, *1*: 292, 299
  quick-bread, *1*: 280
Mucins, *2*: 157
Multiprenyl-menaquinones, *2*: 49–50
Multitude, *2*: 476
Mung bean threads, *1*: 559, 561
Muscle, *2*: 858–859, 860, 864, 876–880
  actin, *2*: 875
  fibers, *2*: 858, 860, 883
  proteins, *2*: 877–879, 883
Muscle proteins, *2*: 777
  gelling polysaccharides, *2*: 779
  solubilization, *2*: 778
Mushroom flavors, *1*: 183
Musts, *1*: 855
Mutants, *2*: 470
Mutations, *2*: 471
Mya arenaria, *2*: 898
Mycotoxins, *1*: 217
Myocomma, *2*: 858
Myofibrillar proteins, *2*: 236, 256, 269, 271, 283
Myofibrils, *2*: 878
Myoglobin, *2*: 244, 256, 262, 315, 858, 882
Myoseptum, *2*: 858
Myotomes, *2*: 858
Myrcene, *1*: 210, 852, 859

(E)-2-Nonenal, *2*: 726
2,6-Nonadienal, *2*: 940
Naringin, *1*: 853, 856
National Marine Fisheries Service (NMFS), *2*: 822
National Shellfish Sanitation Program (NSSP), *2*: 949
National Soft Drink Association, *1*: 421
National Yogurt Association
  live and active culture seal, *2*: 649, 668
Natural breeding, *2*: 461
Natural, processed, and imitation cheese, *1*: 92
Neotame, *1*: 310
Neral, *1*: 852
Neryl acetate, *1*: 853
Neurospora, *1*: 186
Neutrophil, *2*: 389
NFDM, *1*: 310, 619, 697
Nisin, *1*: 604

Nitrate, *2*: 236, 252, 256, 262, 267–268, 282, 316
Nitrate reductase, *2*: 236, 267–268
Nitric oxide, *2*: 244, 315
Nitrite, *1*: 1002, 1005, *2*: 236, 242, 252, 256–257, 262, 267–268, 282, 304, 306, 312, 315–316
Nitrogen moieties, *2*: 480
Nitrogenous compounds, *2*: 905
Nitrosamines, *2*: 257, 273
Nitrosomyoglobin, *2*: 244, 262, 308
NMFS, *2*: 818
Nonanal, *1*: 857
Nonenzymatic browning, *1*: 613, 861
Non-fat dry milk (NFDM), *1*: 310, 605, 697, *2*: 474
Nonfat ice cream, *2*: 595
Nonprotein nitrogen, *2*: 459, 465
Nonstarter lactic acid bacteria (NSLAB), *1*: 572, 589
Nootkatone, *1*: 853, 858
North American freshwater catfish and catfish products, *2*: 849–852
Norvegia, *2*: 499, 501
Novelties, *2*: 630
Novelty category, *2*: 635
  bottom filled, *2*: 640
  cartoon characters, *2*: 643
  extruded, *2*: 636, 644
  fudge bars, *2*: 636
  ice cream sandwich, *2*: 636, 642
  ice pops, *2*: 636
  pudding bars, *2*: 638
  quiescently frozen, *2*: 636–637, 640–641
  stirred, *2*: 640
n-propanol, *1*: 182, 471–472
NRC, *2*: 451
NSLAB, *1*: 572
NSSP, *2*: 947
Nubbin, *2*: 1053
Nubian, *2*: 382
Nuclear magnetic resonance, *1*: 141–142
Nucleotides, *1*: 129, *2*: 284, 287, 289, 471, 858, 905
Nutmeg oil, *1*: 211
Nutraceutical, *1*: 123, 905, *2*: 125
  biological effects, *1*: 914–921
  definition, *1*: 906
  dietary guidance, *1*: 929
  effects on major chronic diseases, *1*: 913
  examples, *1*: 906, 911
  minerals, *2*: 126
    magnesium, *2*: 126–127
    selenium, *2*: 126
    vitamin C, *2*: 127
    vitamin E, *2*: 127
    zinc, *2*: 127
  public education, *1*: 929, 931
  regulation of, *1*: 927–928
  safety issues, *1*: 905, 924
  sources in nature, *1*: 914–922
Nutraceutical products, *2*: 447
Nutrient composition, *2*: 476
Nutrient destruction, *2*: 220
Nutrition, *1*: 610, *2*: 450, 477
  basic, *2*: 450
  human, *2*: 479
Nutrition Labeling Education Act (NLEA), *1*: 283, 940, 949
Nutritional, *2*: 458
  manipulation, *2*: 459
Nutritional benefits of fermented milk, *2*: 105
Nutritional characteristics, milk constituents, *1*: 972
Nutritional education and labeling act, *2*: 593, 623
Nutritional labeling, *1*: 255
Nutritional properties, *2*: 43–45, 47, 49
Nutritive value of ice cream, *2*: 630
Nylon, *1*: 857–858

1,5-Octadien-3-one, *1*: 939
1-Octen-3-ol, *1*: 729, 939
1-Octen-3-one, *1*: 730, 854, 939
25(OH)D_3, *2*: 47–48
β-Ocimene, *1*: 856
O-aminoacetophenone, *1*: 855
O-ethers, *1*: 210
Oakes continuous automatic mixer, *1*: 320
Oakes marshmallow mixer, *1*: 320
Oats, *1*: 922, 1021, 1024, 1026
Oberhasli, *2*: 382
Obesity, *1*: 280, 913–914, 927
Ocean perch, *1*: 930
Octanal, *1*: 853, 857, 861
Octanol-3, *1*: 186

Odors, *2*: 868, 880, 882, 884, 887
Off-flavor. *See* Off-odor
Off-flavors, *1*: 745, *2*: 367
Off-odor, *2*: 235, 237, 311, 315
Ohmic and inductive heating, *1*: 39
Oil recovery under pressure, *1*: 154
Oil, *2*: 463
  canola, *2*: 463
  seed, *2*: 463
Oily-noodles, *1*: 552, 555
Okra, frozen, *2*: 990–991
  styles, *2*: 991
    cut, *2*: 991
    whole, *2*: 991
Oleo-resins, *1*: 212
Oligonucleotides, *2*: 472
Oligosaccharides, *2*: 468, 690
Olive oil
  consumption, *2*: 56
  extra virgin, *2*: 60–62
  heating, *2*: 59
  phytochemicals, *2*: 58
  supplementation, *2*: 62
Omega-3 fatty acids, *1*: 907–908, 924
Onion, *1*: 208, 218
Onion rings, frozen, *2*: 991
  types, *2*: 991
    French fried, *2*: 991
    raw, *2*: 991
On-line flavor concentration measurement methodology, *1*: 393–400
Opioid peptides, *2*: 491–493, 495–496
Optical analysis, *2*: 465
Optical properties, *2*: 366
OR. *See* Oleo-resins
Orange juice, *1*: 847–861
Orangeade, *1*: 135
Ordinary (itohiki) natto, *1*: 73–74
Oregano, *1*: 208–209, 212
Organic acids, *1*: 852, 874
  biosynthesis, *1*: 476
Organic bases, *2*: 932
Organic Foods Production Act of 1990, *1*: 284
Organic milk, *2*: 458
Orotic acid, *2*: 456
Oscillating magnetic fields, *1*: 41
Osmolarity, *2*: 385
Osmotic dehydration, *1*: 871
Osmotic pressure, *2*: 376
Osteoporosis, *1*: 920, *2*: 207–210
Ovens, *1*: 322–324
  band, *1*: 323
  reel, *1*: 322
  rotary rack, *1*: 323
  travelling, *1*: 323
  tunnel, *1*: 324
Overmixing batter, *1*: 315
Ovine, *2*: 470
Oxidation, *2*: 235, 240–243, 245, 256, 261–262, 267, 272–273, 289–290, 305, 388, 390, 858, 877, 879–880, 882, 884, 887–888
Oxidation-reduction potential, *2*: 370
Oxidized flavor, *2*: 468
Oxygen, *1*: 448–449, 454, 473–474, 481, *2*: 858, 879, 882, 886, 887
Oyster
  Eastern oyster, *2*: 898
  Olympia oyster, *2*: 898
  Pacific oyster, *2*: 898
  processing, *2*: 898
Oysters, *2*: 954–955
Oystrea lurida, *2*: 898

2,3-Pentanedione, *1*: 477–478, 488
2-Phenylethanol, *1*: 471–472, 849, 852
2-Phenylethyl acetate, *1*: 474–475
3-Phenylpyruvate, *1*: 473
6-Pentyl-α-pyrone, *1*: 187, 195–196
6-Pentyl-2-pyrone, *1*: 187
6-PP. *See* 6-Pentyl-α-pyrone
α-Pinene, *1*: 174, 848, 852
β-Pinene, *1*: 849
n-Propanol, *1*: 182, 471–472
p-1-menthene-8-thiol, *1*: 851, 853, 855
P-cresol, *2*: 725, 733
p-HPEA and DPHEA, *2*: 59
Package (also packaging), *1*: 583, 585, 590, 609, 627, *2*: 861–868, 870, 874, 879, 886–887
Packages for milk products, *2*: 338
  multilaminated packaging, *2*: 338
  paper-board container, *2*: 342
  plastic packaging, *2*: 338
Packaging, *1*: 42–43, 254, 270, 274, 292, 298, 888, *2*: 242–243, 261, 406, 408–409, 475

anaerobic conditions, *1*: 572
anaerobic pathogenic microorganisms, *1*: 888
fermentative processes, *1*: 888
carbon dioxide, *1*: 268, 271, 274
edible film, *1*: 271
hurdle technology, *1*: 271
MAP, *1*: 271
metal packaging materials, *2*: 904
moisture, *1*: 272
oxygen, *1*: 888–889
paper and card packaging materials, *2*: 904
plastic packaging materials, *2*: 903–904
preservatives, *1*: 271–272
retrogradation, *1*: 271
shelf-life, *1*: 270–271, 273–274
staling, *1*: 271
water activity, *1*: 267, 271–272
Packaging materials, *1*: 53, 252, *2*: 861–862, 867, 869, 874, 886–887
glass, *1*: 228
laminates (paper-based), *1*: 43
metal can, *1*: 43
Packaging, frozen desserts, *2*: 624
heat shock, *2*: 628
manufacturing procedure, *2*: 626
retail display equipment, *2*: 627
retail handling, *2*: 628
storage on retail premises, *2*: 627
transportation, *2*: 627
warehouse equipment, *2*: 627
Packed-bed, *1*: 199
Packed columns, *1*: 158
Packing, *1*: 437
Pandalus
borealis, *2*: 895
dispar, *2*: 895
goniurus, *2*: 895
platyceros, *2*: 895
Paneer, *1*: 645–648
defects, *1*: 647
manufacture, *1*: 647
microstructure, *1*: 648
packaging, *1*: 646
yield, *1*: 647
Pans, cake, *1*: 316
Panulirus
argus, *2*: 897
cuanus, *2*: 897
interruptus, *2*: 897
Paprika, *2*: 1031
carotenoids, *2*: 1032
color, *2*: 1032
dehydration, *2*: 1035
emerging technologies, *2*: 1036
milling, *2*: 1036
processing, *2*: 1034, 1037
quality, *2*: 1040
storage, *2*: 1037, 1039
Paralithodes camschatica, *2*: 897
aztecus, *2*: 893
brasileinsis, *2*: 895
monodon, *2*: 895
Penaeus, *2*: 895
setiferus, *2*: 895
Paralytic shellfish poison (PSP), *2*: 949
Parasite(s), *2*: 950–952
Parathyroid hormone (PTH), *2*: 209
Parity, *2*: 380, 459
Parmigiano Reggiano, *2*: 509
Parsley, *1*: 208–209
Partial coalescence, *1*: 660
Particle size, *1*: 216
Particulate
dried, *2*: 479
Pasta, dried, *1*: 330
cooking quality, *1*: 333
drying, *1*: 338
extrusion, *1*: 335
heat-damage, *1*: 352. *See also* Maillard reaction
process innovations, *1*: 335
quality characteristics before cooking, *1*: 331, 335
raw materials, *1*: 343–344
Pasteurization, *1*: 570, 574–576, 585, 588, 622–623, 728, 857–858, *2*: 388, 394, 404–405, 468
HTST, *2*: 470
LTLT, *2*: 470
Pasteurized, *2*: 457
Pasteurized milk ordinance, *2*: 649, 655, 659
Pasteurizing, *2*: 477
Pastry blend method, *1*: 315
Pathogenic
bacteria, *2*: 458
microorganisms, *2*: 474

Pathogenic bacteria, *1*: 8, 962, 964
Campylobacter jejuni, *1*: 8–9, 884
Cyclospora cayetanensis, *1*: 884
Escherichia coli, *1*: 8, 880
Listeria monocytogenes, *1*: 8, 880
Salmonella enteritidis, *1*: 961
Salmonella spp., *1*: 880, 886
Shigella spp., *1*: 884
Pathogens, *1*: 217, 570, 575, 586, *2*: 86, 91, 94–96
Clostridium botulinum, *2*: 91, 219, 257
Clostridium perfringens, *2*: 769
control, *2*: 26, 256
Escherichia coli, *2*: 157, 769, 809, 869
Listeria monocytogenes, *2*: 91, 142, 504
opportunistic, *2*: 94, 96
Salmonelle, *2*: 509
Pathological, *2*: 457
Peak area, *1*: 395
Peanut butter, *2*: 404
Pears, *1*: 853–854
Peas, frozen, *2*: 991
black-eye, *2*: 992
field, *2*: 992
Pecorino Romano, *2*: 505, 509
Pectin, *2*: 436
high-ester pectin, *2*: 436
low-ester pectin, *2*: 436
protein stabilizing mechanism, *2*: 436
Pectinase, *1*: 188, 856
Pecylomyces, sp., *1*: 186
Penicillium, *1*: 186, 188
Penicillium decumbens, *1*: 183
Penicillium roquefortii, *1*: 181, 183
Pentane-2,3-dione, *1*: 182
Pentanol, *1*: 186
Pentonases, *1*: 188
Pentosans, *1*: 445, 451
Pepper, *1*: 213–215, 218, *2*: 1059
Peppers, frozen sweet, *2*: 992
styles, *2*: 992
diced, *2*: 992
halved, *2*: 992
sliced, *2*: 992
unit, *2*: 992
whole stemmed, *2*: 992
whole unstemmed, *2*: 992
types, *2*: 992
green, *2*: 992
mixed, *2*: 992
red, *2*: 992
Pepsin, *2*: 493–495, 499, 501, 504–509, 723
Peptidases, *1*: 188, *2*: 239, 269–270, 283
Peptides, *1*: 953, 958, 959, *2*: 239, 245, 269–270, 283–286, 288, 856
Peptides, bioactive, *2*: 105–106
Perfumery industries, *1*: 148
Peroxide, *2*: 469
Pervaporation, *1*: 202
Pest, *1*: 225
control, *1*: 229
Pesticide residues, *1*: 217
Pesticides, *2*: 947, 950, 953, 958, 962, 963
PET. *See* Polyethylene terephtalate
pH, *1*: 232–233, 235, 309, 576, 578, 581–582, 590, 600, 608, 619, 621, 623–625, *2*: 218, 220, 239, 241–242, 257, 262, 315, 322, 386, 388, 455, 474, 1046–1047, 1049
alkaline, *2*: 465
Phenolics, *2*: 1112
Phenyl ethanol, *1*: 186
Phenyl ethyl alcohol, *1*: 186
Phenylacetaldehyde, *1*: 855
Phenylketonuria, *2*: 582
Pho, *1*: 540
Phosphatase, *2*: 387
Phosphate, *1*: 729, *2*: 480
Phospholipases. *See* Lipases
Phospholipids, *1*: 654, 726–728, 730, *2*: 461. *See also* Lipids
phosphatidyl choline, *2*: 461
phosphatidyl ethanolamine, *2*: 459
phosphatidyl inositol, *2*: 462
phosphatidyl serine, *2*: 462
Phosphorus (phosphate), *2*: 213, 388, 394
Phosphorylation, *2*: 471
Photoionization detection, *1*: 393–396
Physical characteristics of milk, *2*: 366
acidity and pH, *2*: 368
buffering capacity, *2*: 368
colligative properties, *2*: 376
boiling point, *2*: 377
freezing point, *2*: 376
osmotic pressure, *2*: 376

curd tension, *2*: 375
density and specific gravity, *2*: 373
electrochemical properties, *2*: 370
electrical conductivity, *2*: 370
oxidation reduction potential, *2*: 370
flavor, *2*: 367
foaming, *2*: 374
optical properties, *2*: 366
color, *2*: 366
refractivity, *2*: 366
surface activity, *2*: 374
thermal properties, *2*: 370
effects of heat, *2*: 371
heat capacity, *2*: 370
heat stability, *2*: 371
specific heat, *2*: 371
thermal conductivity, *2*: 371
thermal expansion, *2*: 370
viscosity, *2*: 374
Physical equilibrium, *2*: 350
Physical structure, *2*: 218, 348
Physico-chemical, *2*: 447, 457
properties, *2*: 447, 457, 468, 470
Physiological, *2*: 458
Phytate, *1*: 1006, 1009
Phytochemicals, *1*: 905
areas of future research, *1*: 905, 913
Phytoestrogens (isoflavones), *1*: 915, 923
Phytosterols, *1*: 923
Pickle ingredient, *2*: 1048, 1054
Pickle sizes, *2*: 1049
Pickle(s), *1*: 16, 78–79, *2*: 1046–1047
cured pack, *2*: 1047–1048
dill, *2*: 1047
mustard, *2*: 1047
relish, *2*: 1048
sour, *2*: 1047
sweet, *2*: 1047
fresh pack, *2*: 1048
dietetic, *2*: 1049
dill, *2*: 1049
relish, *2*: 1049
sweet, *2*: 1049
refrigerated, *2*: 1049
styles, *2*: 1046
cut, *2*: 1047
relish, *2*: 1047
sliced crosswise, *2*: 1047
sliced lengthwise, *2*: 1047
whole, *2*: 1046
whole mixed, *2*: 1046
Pickled fruits and vegetables, vegetable sauces and seasonings, and salad dressing, *1*: 96–97
PID. *See* Photoionization detection
Pigment, *2*: 875, 881–882. *See also* Myoglobin
Pineapple, *1*: 833–842
antioxidant capacity, *1*: 821
aroma profile, *1*: 838
classification, *1*: 833
commercial varieties, *1*: 834
Del Monte gold extra sweet, *1*: 833
Smooth Cayenne, *1*: 833
consumption, *1*: 836
cultivation, *1*: 833
flavor components, *1*: 836
Ginaca machine, *1*: 837, 839
chunks, *1*: 837, 839
juice, *1*: 837–839
other pineapple products, *1*: 838–842
pineapple slices, *1*: 837–839
tidbits, *1*: 839
harvest, *1*: 833
brix, *1*: 833
de-crowning, *1*: 835
non-climacteric, *1*: 833
plantation, *1*: 833, 835
nutritional composition, *1*: 836
phenolic content, *1*: 821, 836
processed products, *1*: 837–839
storage, *1*: 833
supply, *1*: 834–835
Piperine, *1*: 212, 217
Plain condensed milk, *2*: 602
Plant, *1*: 225
construction and design, *1*: 227
Plant oil, *2*: 974
Plant sterols, *1*: 1006
Plants and vegetables, *2*: 56
Plasma
antioxidant activity, *2*: 59–60
lycopene, *2*: 58–59, 69
Plasmin, *1*: 727, *2*: 387
Plastic film, *2*: 869
Plate freezers, *1*: 30

Pleurotus, *1*: 183
Plumbing, *1*: 230
Polar bear, *2*: 385
Pollock, *2*: 932, 935, 940
Polyamines, *1*: 994
Polycyclic aromatic hydrocarbons, *1*: 1007
Polydextrose, *1*: 310, *2*: 635, 637
Polyelectrolyte polymer brush, *1*: 653
Polyethylene terephtalate, *1*: 859–860
Polymerase chain reaction (PCR), *2*: 471–472
Polymorphism, *1*: 709, *2*: 458, 464, 470
  protein, *2*: 470
Polyphenolic compounds, *1*: 918
Polyphenols, *1*: 743
Polypropylene, *1*: 859–860
Polysaccharide, *2*: 383
Polysorbates, *2*: 606, 642
Polyunsaturated, *2*: 384
Polyunsaturated fatty acids, *1*: 924, 945
Popularity, *2*: 478
Population, *2*: 449, 451
  human, *2*: 451
Populus nigra, *1*: 167
Porphyromonas gingivalis, *2*: 500, 504, 506
Positional isomers, *2*: 463
  cis-, *2*: 463
  trans-, *2*: 463
Postmenopausal incidences, *1*: 923
Potassium, *1*: 800, 804, *2*: 184, 202, 210, 388
Potassium sorbate, *1*: 857–858
Potato chips, corn chips and similar snacks, *1*: 112–113
Potatoes, *2*: 1014–1018
  conditioned, *2*: 1015
  desugaring, *2*: 1018
  starch content, *2*: 1015
  sugar, *2*: 1018
  varieties, *2*: 1015
Potatoes, frozen French fried, *2*: 992–993, 1014–1018
  fry color, *2*: 1020
  length, *2*: 1022
  manufacture, *2*: 1016–1020
    blanching, *2*: 1018
    by-products, *2*: 1017
    desugaring, *2*: 1018
    frying, *2*: 1018
    peeling, *2*: 1016
    sizing, *2*: 1017
    slicing, *2*: 1017
    trimming, *2*: 1017
    washing, *2*: 1016
  styles, *2*: 993
    crinkle cut, *2*: 993
    dices, *2*: 993
    rissolé, *2*: 993
    slices, *2*: 993
    straight cut, *2*: 993
    strips, *2*: 993
  types, *2*: 993
    institutional, *2*: 993
    retail, *2*: 993
Potatoes, frozen hash browns, *2*: 994
  styles, *2*: 994
    chopped, *2*: 994
    diced, *2*: 994
    shredded, *2*: 994
Potentiator, *1*: 128–129
Pouchitis, *2*: 166
Poultry, *2*: 751–762
  boneless, *2*: 786
  carnosine, *2*: 788
  color, *2*: 756, 759, 789
  color defects, *2*: 756
  consumption, *2*: 784
  cuts composition, *2*: 784
  fatty acids, *2*: 788
  feeding, *2*: 790–791
    tocopherol, *2*: 790
    vitamin E, *2*: 791
  flavor, *2*: 757–758, 760–762, 790–793
    antioxidants, *2*: 791–792
    cholesterol, *2*: 789–790
    lipid oxidation, *2*: 789
    maillard reaction, *2*: 788–789
    off-flavor, *2*: 787, 788
    warmer-over flavor, *2*: 788
  frozen storage, *2*: 792–793
  genotype, *2*: 790
  lipid composition, *2*: 788
    conjugated linoleic acid, *2*: 791
    PUFA, *2*: 791
  lipid oxidation, *2*: 764–765
  marinade ingredients, *2*: 756–766
    acidulants, *2*: 764
    antimicrobials, *2*: 766

antioxidants, *2*: 764
binders, *2*: 763
flavor enhancers, *2*: 762
flavor trends, *2*: 762, 769
formulation, *2*: 757
phosphates, *2*: 759
salt, *2*: 758
species, *2*: 760
sweeteners, *2*: 763–764
marinating systems, *2*: 767
microbiology, *2*: 766, 769
raw materials, *2*: 753
marination, *2*: 751–769
market, *2*: 753–754
MDP, *2*: 785
meat quality, *2*: 754
packaging, *2*: 768, 793
per capita consumption, *2*: 752
pH, *2*: 792
postmortem, *2*: 792
preservation, *2*: 786
processing, *2*: 787
products, *2*: 752
proteins, *2*: 753, 755–756
slaughtering, *2*: 792
spoilage, *2*: 768
storage life, *2*: 786
texture, *2*: 755
water holding capacity, *2*: 755, 758–759
Poultry meat demand, *2*: 775
Poultry slaughtering and processing, *1*: 90–91
Powder, *2*: 404–407
Powdered, *2*: 479
PP. *See* Polypropylene
Prebiotics, *1*: 920, 966, *2*: 110
Precursors, *2*: 43, 47
Premature
infant feeding, *2*: 480
Premium ice cream, *2*: 644
Prepared feed and feed ingredients for animals and fowls except dogs and cats, *1*: 101
Prepared flour mixes and doughs, *1*: 98–99
Prepared fresh or frozen fish and seafoods, *1*: 111–112
Pressing, *1*: 160, 561
Pressure reduced, *2*: 479
Prevention of rickets, *2*: 41, 47
Primer, *2*: 471
Primex, *2*: 932–933
Probiotics, *1*: 920, 958, 960, 963, 1004–1005, *2*: 92–93, 96, 110, 154–158, 162–163, 274, 478, 646, 667
Bacillus cereus, *2*: 68
bifidobacterium sp., *2*: 68, 70, 77
biomarkers, *2*: 68
Enterococcus faecalis, *2*: 68
Escherichia coli, *2*: 68
health benefits, *1*: 961, 966
anticarcinogenesis, *1*: 965
anti-inflammatotory, *1*: 964
lactose digestion, *1*: 963
prevention of diarrhea, *1*: 963
prevention of infectious diseases, *1*: 964
reduction in serum cholesterol, *1*: 965
stimulation of immune system, *1*: 964
urinary tract infections, *1*: 965
Lactobacillus sp., *2*: 68, 70, 76–77
microbes, *2*: 68
products, *2*: 73–75
requirements, *1*: 958
Saccharomyces boulardii, *2*: 68
Saccharomyces cerevisiae, *2*: 68
strains, *2*: 69
survival, *2*: 70, 72
technology, *2*: 71
viability, *2*: 73–74
Probiotic cultured milk products, *2*: 446
Probiotic cultures, *2*: 412, 422
Bififobacteriul bifidum, *2*: 417
Bifidobacterium species, *2*: 412, 423
Lactobacillus acidophilus, *2*: 412, 418, 423
Lactobacillus casei, *2*: 412
Lactobacillus delbreuckii subsp. bulgaricus, *2*: 417–418, 421
Lactobacillus gasseri, *2*: 412
Lactobacillus johnsonti, *2*: 412
Lactobacillus reuteri, *2*: 412
Lactobacillus rhamnosus, *2*: 412
S. thermophilus, *2*: 417–418
therapeutic minimum, *2*: 423
Processing, *2*: 472
characteristics, *2*: 472
Processing and preservation techniques, *1*: 24–41
Processing fluid milk products, *2*: 334–345

Processing procedures, *2*: 599
aseptic processing, *2*: 335, 339–341
direct heating, *2*: 335
filling operation, *2*: 338
high-temperature-short-time (HTST) continuous-flow pasteurizer, *2*: 335
high-temperature-short-time (HTST) pasteurized milk products, *2*: 338
HTST pasteurization process, *2*: 338
in-container sterilization, *2*: 338
maintaining temperature of finished product below 4°C, *2*: 388
milk fat content, *2*: 337
minimal temperature-time relationship, *2*: 336
packaging, *2*: 338
plate heat exchanger, *2*: 335
postpasteurized contamination, *2*: 337
biofilm formation, *2*: 337
processing environment, *2*: 335
receipt of raw milk at processing plant, *2*: 334
steam infusion, *2*: 335
steam injection, *2*: 335
the pasteurization process system, *2*: 335
heat exchangers, *2*: 335
holding tube, *2*: 335
timing pump, *2*: 335
Product decline, *1*: 132
Product development, *1*: 132–145
Product flowcharts, *1*: 11–12
Product formulations, *1*: 11
Product introduction, *1*: 132
Product life cycle, *1*: 135
Production, *2*: 451, 454
record, *2*: 452
Prolactin, *2*: 467
Proline, *1*: 975
Proofing, *1*: 297
Propanoic acid, *1*: 849
Propanol, *1*: 186
Propionibacteria, *1*: 181
Propionic acid, *1*: 271
Propyl acetate, *1*: 186
Propyl butanoate, *1*: 856–857
Proteases, *1*: 188, 600, 854, *2*: 480, 540. *See also* Proteinases
heat stability, *2*: 546
types, *2*: 540
Protective cultures, *2*: 93
Protein, *1*: 930, 991, 996, 1001, 1004, *2*: 381, 458, 464–465, 857–858, 878–879, 883, 888
amino acids, *1*: 991
amino acid profile, *2*: 577
casein, *2*: 558–587
coagulable, *2*: 467
digestibility, *2*: 577
emulsions, *2*: 573
foams, *2*: 573
gelation, *2*: 572
hydration, *2*: 575
loci, *2*: 471
major, *2*: 471
minor, *2*: 467
modifier, *2*: 465
nitrogen conversion factor, *2*: 580
plant-based, *1*: 1004
polymorphism, *2*: 470, 472
solubility, *2*: 570
surface activity, *2*: 572
whey, *2*: 465, 471
Protein hydrolysates, *2*: 538
analysis, *2*: 540
applications
bioactive peptides source, *2*: 539, 547–552
hypoallergenic infant formulas, *2*: 539, 547, 548–552
nutrition support, *2*: 547, 550
nutritional/protein supplements, *2*: 547–548
definition, *2*: 539
parameters
AN/TN ratio, *2*: 541, 543, 550
degree of hydrolysis (DH), *2*: 541, 550
molecular weight distribution, *2*: 542, 548
peptide chain length (PCL), *2*: 542
production, *2*: 544
post-hydrolysis processes, *2*: 546–548
properties, *2*: 546, 550
Proteinase K, *2*: 502
Proteinases, *2*: 269
Proteins in milk, *2*: 693
analytical techniques, *2*: 698
human vs bovine, *2*: 695
plant proteins for infant formulas, *2*: 698

Proteolysis, *1*: 572–573, 576, 584, 605, 976, *2*: 239, 245, 262, 270–271, 283–285, 296, 322, 491, 501, 502
Proteolysis and related reactions, *2*: 722
Proteose, *2*: 465
  activity, *2*: 467
  peptone, *2*: 465
Proving, *1*: 265–268, 274
  fermentation, *1*: 266–268
  humidity, *1*: 268
  proving temperature, *1*: 268
  volume expansion, *1*: 268
  yeast, *1*: 262–263, 266–268, 271, 273
Provolone, *1*: 188
Proximate, *2*: 458
  composition, *2*: 458
PSE, *2*: 235
Pseudocereals, *1*: 1022–1023, 1025
  amaranth, *1*: 1023–1024
  buckwheat, *1*: 1023
  quinoa, *1*: 1023
PSP, *2*: 949
Psychrotrophic bacteria, *1*: 570
Psyllium, *1*: 922
Pulsed electric field processing, *1*: 125
Pulsed electric fields (PEF), *1*: 40
Pulsed light technology, *1*: 41
Pulsed X-ray, *1*: 42
Pumping, *1*: 23
Putrescine. *See* Amines
p-vinylguaiacol, *1*: 861
Pyrazine, *1*: 187, 400–401, 406
Pyroglutamate, *1*: 476
Pyrones, *2*: 726
Pyruvate, *1*: 471, 476

Quality, *1*: 421, 425, *2*: 881
Quality control operation(s), *1*: 225, 232
Quality evaluation, *2*: 1023–1027
  frozen French fried potatoes, *2*: 1023
    flavor, *2*: 1023
    fry color, *2*: 1021–1023
    uniformity, *2*: 1024
Quality requirements, *2*: 1056–1059
Quantitative descriptive analysis, *1*: 143
Quantitative variations, *2*: 470
Quark, *1*: 636
Queso blanco, *1*: 648
Quick mix methods, *1*: 315
Quinoa, *1*: 1023

Rabbit, *2*: 381
Radiation, *2*: 868
Radiation heat, *1*: 322
Raisins, *1*: 12
Rajbhog, *1*: 643
Ramen, *1*: 550
Rancid, *2*: 389, 394
Rancidity, *2*: 241, 256, 323, 877, 879–880, 885–886
Randomized Controlled Trials (RCTs), *2*: 159, 178
Rasmalai, *1*: 643
Rasogolla, *1*: 639–640
  cooking medium, *1*: 641
  packaging, *1*: 646
  sugar syrup, *1*: 641
  yield, *1*: 642
Rat, *2*: 390
Raw fish, *2*: 965
  brining, *2*: 965
Raw materials handling, *1*: 20
Raw milk, *2*: 89, 96
Raw milk quality, *2*: 332–334
  added water, *2*: 334
  antibiotic residues, *2*: 333–334
  bacterial limit, *2*: 333
  caustic cleaner, *2*: 333
  farm bulk tank operation, *2*: 334
  grade A standards (USHHS), *2*: 332
  mastitis, *2*: 333
  preliminary incubation count, *2*: 334
  sediment grade standard, *2*: 333
  somatic cell count, *2*: 333
  standard methods for the examination of dairy products, *2*: 331
  temperature standard, *2*: 333
RDA, *2*: 41–42
  vitamin A, *2*: 43–44
  vitamin D, *2*: 47
  vitamin E, *2*: 46
  vitamin K, *2*: 50
Recombination, *2*: 472
Rectification, *1*: 159
Redox potential, *2*: 219
Redox system, *2*: 49

Reduced-fat, *1*: 311, 573, 575
Reel oven, *1*: 322
Refractive index, *2*: 472
Refractivity, *2*: 367
Refrigeration, *2*: 861, 865, 871–872
Refrigerators, *1*: 27, 79
Regulation of genes, *2*: 46–49
Rehydration, *1*: 868
Reindeer, *2*: 380–381
Renal stones, *2*: 202, 210
Rennet, *2*: 394–395
Rennetability, *2*: 470
Renneting, *2*: 472
  property, *2*: 472
  time, *2*: 473
Repercussions, *2*: 472
Restrictive environment, *2*: 156
Retarded dough, *1*: 69
Retinoids, *2*: 43–45
Retorts (pressure cookers), *1*: 26
Reverse R & D, *1*: 133
Rework, *1*: 226
Rhizopus, *1*: 76
Rhizopus oryzae, *1*: 195
Ribonuclease, *2*: 467
  pancreatic, *2*: 468
Rice milling, *1*: 528
Rice noodle (fen), *1*: 540, 556
Rice sticks, *1*: 557
Rice, *1*: 523
  bran, *1*: 533
  brown, *1*: 526
    minerals, *1*: 526
  flour, *1*: 531
    breads, *1*: 532
      enzymes, *1*: 532
      hydrocolloids, *1*: 532
  hulls, *1*: 533
  parboiled, *1*: 526
  processing, *1*: 525
  vitamins, *1*: 526–527, 529
    enrichment, *1*: 529
Rice-based asian noodles, *1*: 556–558
Ricinoleic acid, *1*: 172
Ricinus communis, *1*: 197
Ricotta cheese, *1*: 638
  manufacture, *1*: 638
Rigor mortis, *2*: 877
Ripening (of meat products), *2*: 237, 252
Ripening, *1*: 569, 572, 576, 578, 580, 584, 588, 880–881, *2*: 476
  climacteric fruits, *1*: 881
  ethylene, *1*: 881–884, 889
Ripples/variegates, *2*: 618
RNA splicing, *2*: 470
Roasted coffee, *1*: 112
Roasters, *1*: 26
Rocking-drum bioreactor, *1*: 199
Romano, *1*: 188, *2*: 389, 394
Root beer, *2*: 404, 406
Roquefort, *2*: 389, 394
Rosemary, *1*: 209, 212
Rotary rack oven, *1*: 323
Rotating drum, *1*: 199
Rotation, *2*: 479
Roughage, *2*: 459
Rue oil, *1*: 209
Rumen, *2*: 470
Ruminant, *2*: 383

α-Sinenasal, *1*: 852
β-Sinenasal, *1*: 852
Saanen, *2*: 382
Saccharin, *1*: 310
Saccharomyces carlsbergensis, *1*: 183
Saccharomyces cerevisiae, *1*: 175, 183, *2*: 495, 497
Safe moisture level, *1*: 226
Safety, *1*: 575, 583–584, 586, *2*: 871–872
Safrole, *1*: 211
Sage, *1*: 212
Sai fen, *1*: 559
Saimen, *1*: 545
Sales, *1*: 618, 633, *2*: 635
Salinometer, *2*: 965
Salmon, *2*: 858, 882, 885
Salmon control plan, *2*: 949
Salmonella, *1*: 8, 217, *2*: 509
Salt, *1*: 290, 296, 569, 572, 583, 585, 618–620, *2*: 236–239, 242–243, 256, 282, 287, 306, 311, 316, 322, 867, 876, 885–887 (*See also* Sodium, Salting)
  salt in moisture ratio, *1*: 583
Salt equilibria, *2*: 474
Salt stations, *2*: 1056
Salt stock tanks, *2*: 1056
Salted and roasted nuts and seeds, *1*: 106

Salting, *2*: 236–237, 316, 477
Salty yogurt (laben), *2*: 433
Sample unit size, *2*: 1054
Sampling schedule for color containing products, *1*: 253
Sandesh, *1*: 639–640
  physico-chemical attributes, *1*: 644
Sanitation, *1*: 229, 240, *2*: 218, 949–950, 952–958, 1020
Sanitizer, *2*: 859
Sanitizer(s), *2*: 955, 960
Sanitizing treatments, *1*: 881
  chlorine, *1*: 881
  chlorine dioxide, *1*: 881
  hydrogen peroxide, *1*: 798, 885
  organic acids, *1*: 880
  ozone, *1*: 885
  ultraviolet light, *1*: 885
Sanitizing, *1*: 226, 229, *2*: 951–952, 954, 957, 959, 962, 965
  agents, *1*: 228
  food-contact surfaces, *1*: 229
  non-food contact surfaces, *1*: 229
Sapid compounds, *1*: 209–212
Saponification value, *2*: 472
Sassafras root oil, *1*: 211
Satiety, *2*: 583
Saturated, *2*: 384
Sauerkraut, *1*: 78
Sausages, *2*: 252–256, 282, 285, 288, 290–296, 304
  basic steps in dry sausage processing, *1*: 65
  cooking, *2*: 261
  drying, *2*: 261
  emulsification, *2*: 260
  fermentation, *2*: 260
  processing, *2*: 257–262
  ripening, *2*: 252–254, 258, 261
  traditional, *2*: 252
  types of
    Bologna, *2*: 254, 256
    chorizo, *2*: 254–255, 267
    cooked sausages, *2*: 254–256
    dry fermented sausages, *2*: 254, 315–316
    frankfurter, *2*: 254, 256, 282, 291–292, 296, 308–312
    french, *2*: 252, 255
    fresh, *2*: 253
    German, *2*: 255
    hot-dog, *2*: 254–256
    Hungarian, *2*: 255
    Italian, *2*: 255
    Lebanon Bologna, *2*: 254
    Longaniza, *2*: 254
    pepperoni, *2*: 254–255
    poultry, *2*: 256
    raw fermented, *2*: 253–254
    salami, *2*: 252, 254–255, 316
    Salchichón, *2*: 252, 254–255
    semidry sausages, *2*: 254
    summer sausage, *2*: 254–255
    wiener, *2*: 254, 256
Sausages and other prepared meat products, *1*: 89–90
Savory flavors, *2*: 931
Savory noodles, *1*: 552
Scaling, *1*: 285, 295, 297
Scallops, *2*: 932, 958, 959–960
Scandinavian sour milk, *2*: 432
Scandinavian sour milk products, *2*: 415–416
Scottish blended whisky, *1*: 516
  patent still (Coffey still), *1*: 515
    analyzer, *1*: 515
    rectifier, *1*: 515
Scottish grain whisky, *1*: 515
Scottish malt whisky, *1*: 508
  angel's share, *1*: 513
  Bourbon, *1*: 513
  Campbeltown, *1*: 515
  cask, *1*: 513
    charring, *1*: 513
  distillation, *1*: 510
    congeneric, *1*: 512
    demisting test, *1*: 512
    feints (tail), *1*: 513
    feints receiver, *1*: 513
    foreshot (head), *1*: 512
    hearts (middle cut), *1*: 513
    low wine, *1*: 512
    low wine charger, *1*: 512
    low wine still, *1*: 512
    new pot (new spirit), *1*: 513
    spent lees, *1*: 513
    spirit, *1*: 513
    spirit receiver, *1*: 513
    wash still, *1*: 512

Scottish malt whisky (*Continued*)
draff, *1*: 510
dunnage warehouse, *1*: 513
highland malts, *1*: 515
Islays, *1*: 515
kiln, *1*: 508
lowland malts, *1*: 515
malting, *1*: 508
germination, *1*: 508
steeping, *1*: 508
maltster, *1*: 509
mash tun, *1*: 509–510
peat, *1*: 509
pot still, *1*: 510
condenser, *1*: 512
lyne arm, *1*: 510
worm, *1*: 512
sherry, *1*: 513
wash, *1*: 515
washback, *1*: 510
wort, *1*: 510
Scrap candy, *1*: 699
Scraped-surface freezers, *1*: 31
Seafood byproducts, *2*: 930–931
Seafood byproducts flavorants, *2*: 929–940
Seafood byproducts flavors, *2*: 932
amino acids, *2*: 935
cucumber and green-like odors, *2*: 940
enzymatic hydrolysis production, *2*: 933
malty, caramel, and sweet-like odors, *2*: 940
mushroom-like odor, *2*: 941
other odors, *2*: 947
peptides, *2*: 936
popcorn-like odor, *2*: 938
potato-like odor, *2*: 938
volatiles, *2*: 937
Seafoods, frozen, *2*: 818
fish fillet blocks, *2*: 826–828
fresh and shrimp, *2*: 840–843
fried fish portions, *2*: 839–840
fried fish sticks, *2*: 837–839
halibut steaks, *2*: 823–824
headless dressed whiting, *2*: 822–823
minced fish blocks, *2*: 829–832
North American freshwater catfish and catfish products, *2*: 849–852
raw breaded fish portions, *2*: 835–837
raw breaded fish sticks, *2*: 833–835
raw breaded scallops and fried scallops, *2*: 847–849
raw breaded shrimp, *2*: 843–846
raw fish portions, *2*: 832–833
raw scallops, *2*: 846–847
salmon steaks, *2*: 824–825
Season, *2*: 457, 459, 461
Seasonal, *2*: 457
deviation, *2*: 461
Seasoner, *1*: 128
Seasoning blends, *1*: 219
Seasonings, *2*: 257
Secondary metabolism, *1*: 180
Sediment, *2*: 455
Selenium, *1*: 924, 993, *2*: 456
Semidry-fermented sausages. *See* Fermented sausages
Semolina, *1*: 329–330, 333
rheological characteristics, *1*: 332
Seniors nutrition products, *1*: 969
Sensorial simulation, *1*: 145
Sensory
descriptive terms, *2*: 306–323
quality, *2*: 240–241, 243–244, 262, 273, 302–322
Sensory analysis, *1*: 143
Separating, *1*: 22
Separation, *1*: 622, 664–665
Separation technique, *1*: 157
Separators, *2*: 479
Septoria nodorum, *1*: 187
Sequenced, *2*: 463
Serum albumin, *2*: 463
bovine, *2*: 463
Serum protein, *2*: 463
Serum solids, *2*: 549
Sesquiterpene, *1*: 210, 852
S-ethers, *1*: 210
Sewage disposal, *1*: 230
Sex odor, *1*: 123
SFA1 gene, *1*: 473
Shallot, *1*: 208
Shape, pickles, *2*: 1051
crooked, *2*: 1051
curved, *2*: 1051
defect, *2*: 1051
diameter, *2*: 1051
end cut, *2*: 1052

Sheath, *2*: 858
Sheep, *2*: 380, 385–387, 389, 392, 450, 454
Shelf-life, *1*: 287, 298, 584, 600, 611, *2*: 218, 407–409, 879–880, 884–887
Shelf line, *2*: 470
Shelf stability, *2*: 881, 887
Shellfish, *1*: 21, 29
Shellfish freezing
  by-products of frozen shellfish, *2*: 908–909
  effects of freezing on physical properties, *2*: 904–907
  freezing, *2*: 894
  freezing methods, *2*: 899–902
  glazing, *2*: 902
  harvesting, *2*: 895–898
  microbiological quality, *2*: 907–908
  nutritional values, *2*: 896
  packaging, *2*: 903
  quality control and assurance, *2*: 909–912
  thawing, *2*: 902–903
  varieties, *2*: 896–898
Sherbet, *2*: 595, 641, 644
Shiitake, *1*: 183
Shirataki, *1*: 561
Short-chain fatty acids, *2*: 161
Shortened style cakes, *1*: 314
Shortening, *1*: 311
Shortening, table oils margarine and other edible fats and oils, not elsewhere classified, *1*: 108
Shrimp, *1*: 30–31, *2*: 882–883, *2*: 960–964
  aquacultured shrimp, *2*: 895
  cold water shrimp, *2*: 895
  freezing requirement, *2*: 896
  melanosis, *2*: 905
  mushiness, *2*: 906
  saltwater shrimp, *2*: 895
  warm water shrimp, *2*: 895
Signaling, *2*: 158
Silicon carbide, *1*: 489
Single stage (dump method), *1*: 315
Single-service articles, *1*: 229
Size distribution of milk fat globules, *2*: 353
Skyr, *2*: 420, 424, 425
Small entity compliance guide (SECG), *1*: 929
Smell, *1*: 207
S-methylmethionine sulfonium, *1*: 855
SmF. *See* Submerged fermentation
Smoke. *See* Smoking
Smoked fish, *2*: 964–967
Smoking, *2*: 237, 240–241, 252, 254–255, 257, 260–261, 282, 290, 296
SNIF/NMR, *1*: 215
Sniff testing, *1*: 143
Soba, *1*: 377
Soda pops, *1*: 420. *See also* Carbonated soft drinks
Sodium, *1*: 729, 737, 895, *2*: 161, 210, 1051
Sodium alginate, *2*: 404, 406
Sodium aluminum sulfate, *1*: 312
Sodium carboxymethyl cellulose, *2*: 605
Sodium caseinate, *2*: 494, 501, 509
Sodium chloride. *See also* Salt *1*: 290
Soft drinks, *1*: 420. *See also* Carbonated soft drinks
Softening, *1*: 777
  calcium chloride, *1*: 786
  calcium lactate, *1*: 888
  polygalacturonase, *1*: 784
  proteolytic enzymes, *1*: 883
Solid fermentation, *1*: 197–198
Solid-liquid extraction, *1*: 150
Solid-not-fat (NSF), *2*: 458, 459
Solid-phase micro extraction, *1*: 152
Solids, *2*: 457, 637, 639, 644
  total, *2*: 459
Solid-state fermentation, *1*: 197–198
Solvent extraction/AEDA, *1*: 155, 212
Somatic cells, *1*: 571, *2*: 389, 394–395
Somatic cell counts, *2*: 457
Somen, *1*: 541–542, 544
Sorbet, *2*: 596, 641
Sorbic acid, *1*: 604, 614
Sorbitol, *2*: 637
Sorghum, *1*: 1025–1026
Sotolon, *2*: 726–727
Sources of vitamins, *2*: 41
  vitamin A, *2*: 43
  vitamin D, *2*: 47
  vitamin E, *2*: 46
  vitamin K, *2*: 50
Sour cream, *2*: 478, 521
  analysis, *2*: 532
  chemical and physical background, *2*: 520
  consumer safety, *2*: 532
  cultures, *2*: 524

Sour cream (*Continued*)
economical significance, *2*: 522
history, *2*: 520
ingredients and additives, *2*: 523
legal definitions, *2*: 521
manufacturing, *2*: 526
packaging, *2*: 530
quality, *2*: 532
usage, *2*: 531
Sour dip, *2*: 478
Sour milk manufacturing, *1*: 60–62
Sourdough, *1*: 361, 367, 1023–1024
fermentation, *1*: 1023
starter cultures, *1*: 572
types, *1*: 361
Soxhlet extractor, *1*: 153
Soy, *2*: 393
Soy proteins, *2*: 698
Soy sauce, *1*: 73
Soybean oil mills, *1*: 106–107
Soybeans, *1*: 922–923
Soymilk, *2*: 1064–1065
grinding, *2*: 1067
heating and separation, *2*: 1068
preparation, *2*: 1065
quality, *2*: 1081–1082
solids content and pH, *2*: 1070
Spearmint oil, *1*: 210
Specialty gourmet, *2*: 451
Species, *2*: 450, 456, 459
Specific gravity, *2*: 373, 1016
Specific heat, *2*: 371
Spent grains, *1*: 487
Sphingomyelin, *2*: 461
Spices, *1*: 205–220, *2*: 255, 257, 287, 290, 294–295, 311, 316
in the food industry, *1*: 217–219
Spice blends, *1*: 219
Spice trade, *1*: 213–215
Spisulla solidissima, *2*: 898
Spoilage, *2*: 236, 243, 260, 857, 859, 877, 880–882
Sponge cake, yellow, *1*: 318
Spontaneous lipolysis, *2*: 463
Sporidioolus salmonicolor, *1*: 201
Sporobolomyces odorus, *1*: 187
Sports nutrition, *2*: 582
Sports nutrition products, *1*: 969
Spray-drying, *1*: 201–202
Spreading, *1*: 719
SSF. *See* Solid-state fermentation
St. John's wort, *1*: 926–927
Stability, *2*: 868–876, 881, 884, 886–887
Stabilizers, *1*: 620, *2*: 404–407, 479, 596, 604, 637, 642
Stage of lactation, *2*: 459, 468
Standardization, *2*: 474, 477
Staphylococcus aureus, *2*: 333, 500, 504–505, 508–509
Staphylococcus carnosus, *2*: 500, 504–506
Starch, *1*: 449–450, *2*: 245, 262
Starch noodles, *1*: 559
Starch-based noodles, *1*: 559–561
Starter adjuncts, *2*: 92–93
Starter culture. *See* Culture
Starter cultures, *2*: 86–89, 92, 96, 256–258, 266–270, 285
Static headspace-olfactometry, *2*: 732
Steam, *1*: 312
Steam distillation, *1*: 160–162, 211, 410–411
Steamed bread (Mantou), *1*: 70–71
Steam-heated, *2*: 479
Steatorrhea, *2*: 480
Sterilized, *2*: 478
Sterols, *1*: 333
Stick, *2*: 635–636
Stinky tofu, *1*: 75–76
Stirred aerated bed, *1*: 199
Stoke's law, *2*: 363
Stomach acid, *2*: 478
Storage, *1*: 667, 880, 887, 889, 891, *2*: 218, 478, 869, 872
Storage condition, *1*: 574, 667, 686, 691
Str. creamosis, *2*: 437, 444
Str. lactis, *2*: 437, 444
Str. thermophilus, *2*: 440
Strained yogurt (labneh), *2*: 443
Strawberries, *1*: 793–804
average production, *1*: 795
demand, *1*: 795
physicochemical, phytonutrients, and nutritional qualities, *1*: 808–811
anthocyanins, *1*: 798
antioxidant capacity, *1*: 799, 808–809
antiproliferative properties, *1*: 799
nutritional quality, *1*: 800

pectins and cell wall enzymes, *1*: 797
phenolic compounds, *1*: 798
strawberry flavor, *1*: 797
sugars and organic acids, *1*: 796
strawberry products, *1*: 800–804
freeze dried, *1*: 804
frozen, *1*: 800
infused and stabilized, *1*: 803
infused dried, *1*: 804
strawberry juice, *1*: 801
strawberry puree, *1*: 800
strawberry varieties and postharvest handling, *1*: 795–796
Strawberries and blueberries: phytonutrients and products, *1*: 793–812
Strecker, *2*: 245, 270, 287–289, 296
Strecker aldehydes, *1*: 464
Strecker degradation, *1*: 185, 861
Strecker reaction, *2*: 932
Strep. diacetylactis, *2*: 437, 444
Streptococci, *1*: 181
Streptococcus mutans, *2*: 490, 504, 506
Streptococcus pyogenes, *2*: 504
Streptococus lactis, *1*: 184
Streptococcus thermophilus, *1*: 961, *2*: 103, 412, 477, 649, 652, 659, 664, 666, 669, 652–653, 656, 661
Stripping, *1*: 149, 525
Stripping using water vapor, *1*: 149
Structure of vitamins
vitamin A, *2*: 42–44
vitamin D, *2*: 47–48
vitamin E, *2*: 45, 47
vitamin K, *2*: 49–50
Stuffing, *2*: 258
Submerged fermentation, *1*: 196
Subsistence, *2*: 450
Substitution, *2*: 470–471
Substrate, *2*: 465
Succinate dehydrogenase, *1*: 476
Succinic acid, *1*: 885
Sucrase-isomaltase, *2*: 168
Sucrose, *1*: 286, 468, 852–853, *2*: 479, 603, 637–639, 644
Sufu (fermented soy cheese), *1*: 75–76
Sugar, *1*: 286, 431–432, 926, *2*: 636
beet sugar, *1*: 104, 431
brown, *1*: 310
cane sugar, *1*: 103–104, 252, 308
granulated, *1*: 314
handling and treatment at beverage plant, *1*: 432
liquid sucrose, *1*: 431, 435
medium Invert sugar, *1*: 431–432, 435
Sugar alcohols, *2*: 602
Sugar and confectionery products, *1*: 103–106
Sugar replacers, *1*: 266, 281, 287, 302
Sugar syrup, *2*: 478
Sugar-free coating, *1*: 689–690
Sulfite reductase, *1*: 481
Sulphite, *1*: 480–481
Supercooling, *2*: 860, 862
Supercritical extraction, *1*: 167
Supercritical fluid extraction, *1*: 152–154, 162
Supervision, *1*: 227
Supplementary feeding, *2*: 454
Supplementation, *2*: 463
Supplies, *1*: 425–426
Surface activity, *2*: 375
Surface ripened, *2*: 476
Surface tension, *2*: 478
Surimi-based products, *2*: 933
Sweet caramel, *2*: 478
Sweet fennel, *1*: 210
Sweeteners, *1*: 286, 295, 429–432, *2*: 402–404, 479, 602, 637
corn syrup, *1*: 286, 295
dextrose, *1*: 295
fruit juice, *1*: 286
honey, *1*: 286, 295
maple sugar, *1*: 286
non-nutritive, *2*: 604
nutritive, *2*: 602
Swiss cheese manufacturing, *1*: 55, 57
Swordfish, *2*: 882
Symbiotics, 1: 920
lactic acid bacteria, *2*: 478
Synbiotics, *1*: 966, *2*: 71, 106
Syneresis, *1*: 666–667
Syneresis, yogurt, *2*: 652–653, 656, 661
Synergy, *2*: 44–46
Syrup, *2*: 636–637, 639, 644

2-*Trans*-hexenal, *1*: 858, 860
α-Terpineol, *1*: 173, 857, 861
γ-Terpinene, *1*: 853
TAG. *See* Triacylglycerol
Tangerine flavor, *1*: 853
Tanks, *1*: 26
Tarragon, *1*: 210, 218
Taste, *1*: 207, *2*: 236, 239, 256
Taste compounds in milk and milk products, *2*: 736
Taurine, *2*: 858
Tea, *1*: 918
Tempe (tempeh), *1*: 76–77
Temperate climate, *2*: 460
Temperature, *1*: 880–881, 891–892, *2*: 884–885
Tempering of chocolate, *1*: 684–686
Tenderness. *See* Texture
Terpenes, *1*: 187, 210, 852
Terpenyl acetate, *1*: 210
Terpin-4-ol, *1*: 857
Terpineols, *1*: 210
Tert-butylbenzene, *1*: 392–408
Texture, *1*: 293, *2*: 237, 239, 243–245, 262, 306–321, 823–824, 828–829, 831–832, 883, 1053
Texture and physical appearance, *2*: 924
T_g. *See* Glass transition temperature
Thaw, *2*: 873–874, 881
Therapeutic values, *2*: 479
Thermal conductivity, *2*: 371
Thermal degradation, *1*: 857
Thermal expansion, *2*: 370
Thermal processing, *2*: 225, 227
  aseptic processing, *2*: 229
  canning, *2*: 229
  cooking, *2*: 218, 225
  D-value, *2*: 223
  F-value, *2*: 224
  pasteurization, *2*: 225
  radiation, *2*: 222
  scalding, *2*: 225
  sterilization, *2*: 218, 225
  Z-value, *2*: 224
Thermal properties, *2*: 370
Thermally-induced changes, *2*: 717
Thermolysin, *2*: 494, 500, 502
Thiamin, *1*: 861
Thiazole, *2*: 717, 939–941
Thin film, *2*: 479
Thiocyanate, *2*: 388
Threonine, *1*: 478, 484
Threshold, *2*: 464
Thrombin, *2*: 494
Thyme, *1*: 167, 209
Thymus vulgaris, *1*: 167
Time-temperature, *2*: 955, 958, 960
Tiquira, *1*: 186
Tissue, *2*: 857–858, 860–881
Titratable acidity, *1*: 619, 624, *2*: 478
Titration, *2*: 465
  curve, *2*: 465
TMA. *See* Trimethylamine, *2*: 942
TMAO. *See* Trimethyl amine oxide, *2*: 858
Tocopherol, *2*: 45–47, 51
Tocotrienol, *2*: 45
Tofu, *2*: 1065–1067
  cultivar/material effect, *2*: 1074
    chemical composition, *2*: 1081
  identity-preserved, *2*: 1074, 1081
    size and hilum color, *2*: 1081
    soybean quality, *2*: 1081–1082
  isoflavones, *2*: 1079
  lipoxygenases, *2*: 1080
  mechanism of curd formation, *2*: 1080–1081
    lipids, *2*: 1077
    phospholipids, *2*: 1077
    phytate, *2*: 1078
    protein charges, *2*: 1081
    quality, *2*: 1082
  saponins, *2*: 1079
  soy storage protein, *2*: 1076–1077
    beta-conglycinin (7S), *2*: 1077
    gelation, *2*: 1078
    glycinin (11S), *2*: 1077
    ratio of 11S/7S, *2*: 1077
  storage effect, *2*: 1075–1076, 1082
  type, *2*: 1071
Tofu processing, *2*: 1071–1072
  coagulation, *2*: 1070
    coagulant concentration, *2*: 1072
    coagulation method, *2*: 1073
    coagulation temperature, *2*: 1072
    soymilk concentration, *2*: 1070
    type of coagulants, *2*: 1071

pilot-scale method, *2*: 1082
pressing, *2*: 1074
small-scale method, *2*: 1082
soaking, *2*: 1065
Toggenburg, *2*: 382
Tomatoes, *1*: 916, *2*: 58–62, 1109
dehydration, *2*: 1110
dried, *2*: 1109, 1113
nutritional composition of, *2*: 1111–1112
solar drying of, *2*: 1115
spray drying of, *2*: 1117–1118
sun drying of, *2*: 1115
Tomato catsup, *2*: 1105–1106
Tomato concentrate, *2*: 1101–1104
aseptic processing, *2*: 1102
break, *2*: 1101
color, *2*: 1103
pectinmethylesterase, *2*: 1101
polygalacturonase, *2*: 1101
quality standards, *2*: 1103
Tomato juice, *2*: 1104–1105
aseptic processing, *2*: 1105
break, *2*: 1104
Tomato processing, *2*: 1091–1107
biology, *2*: 1093
lycopene, *2*: 1092
lycopersicon esculentum, *2*: 1093
nutritional value, *2*: 1095
Tonnage, *2*: 451
Total solids, *2*: 595
Total volatile basic nitrogen (TVB-N), *2*: 909
Toxicity, *2*: 41–42, 44
Toxins, *2*: 880
Trace elements, *1*: 922
Traditional lactic bacteria, *2*: 412
mesophilic lactic bacteria, *2*: 412
thermophilic lactic bacteria, *2*: 412
Trametes odorata, *1*: 187
Trans-2-hexenal, *1*: 856, 860
Trans-2-nonenal, *1*: 481, 855
Transamination, *1*: 472, *2*: 268, 270
Transcriptional regulators, *2*: 44
Transfer of heat, *1*: 322
Transferrin, *2*: 467
Transformation, *2*: 479
Transglutaminase, *1*: 1027
Translocation, *2*: 157
Transport, *1*: 667
Trans-resveratrol, *1*: 919
Travelling ovens, *1*: 323
Tray, *1*: 199
Tray columns, *1*: 158
Trends, *1*: 273
automation, *1*: 274
frozen dough, *1*: 273–274
frozen par-baked products, *1*: 273
functional food, *1*: 273
health, *1*: 262, 273
optimal control, *1*: 274
Triacylglycerides, *2*: 382
Triacylglycerol, *1*: 653
Triacylglycerols. *See* lipids
Trichoderma, *1*: 188
Trichoderma harzianum, *1*: 187, 195
Trichoderma viridae, *1*: 183, 185, 187, 196
Trichosporon, *1*: 181
Triglyceride. *See* Triacylglycerol
Triglycerides, *1*: 209, 718, *2*: 472, 480
Trimethylamine (TMA), *1*: 940, *2*: 909
Trimethylamine oxide (TMAO), *2*: 858, 879, 883–886, 905
Tripeptidyl peptidase. *See* Peptidases
Triticum, *1*: 1021
Trub, *1*: 447, 466–467
Truffles, *1*: 183
Trypsin, *2*: 491, 493–494, 496, 498–500, 502, 504, 507, 509
Tryptophol, *1*: 471–472
Tuber sp., *1*: 183
Tuna, *2*: 857, 882
Tunnel, *2*: 865, 867
Tunnel ovens, *1*: 26, 324
Turmeric, *1*: 217
Tyramine. *See* Amines
Tyrosine, *2*: 237, 239, 270, 273
Tyrosol, *1*: 471–472

U.S. Code of Federal Regulations (CFR), *1*: 224
U.S. Compressed Gas Association (CGA), *1*: 433
quality verification levels, *1*: 433
U.S. FDA Food Additives Status List (FASL), *1*: 252
U.S. FDA United Registartion and Listing System ((FURLS), *1*: 246
U.S. Food and Drug Administration (FDA), *1*: 224, *2*: 1046, 1049

U.S. Occupational Safety and Health Administration (OSHA), *1*: 434
U.S. Standard Industrial Classification (SIC), *1*: 87
  SIC major industrial group 20 (foods and kindred products), *1*: 88
  SIC manual, *1*: 87
Udon, *1*: 543, 545
UHT, *2*: 454, 470, 474
UHT treated milk, *1*: 743
UHT-treatment, *1*: 735
Ulcers, *2*: 480
Ultrafiltration (UF), *1*: 200, 575, *2*: 416
Ultrasound, *1*: 42
Ultrastructure
  buckwheat, *1*: 376–378
  dried pasta, *1*: 333
  fresh pasta, *1*: 339
Ultraviolet light, *1*: 41
Umbelliferae, *1*: 208
Umbelliferous fruits, *1*: 122
Underdeveloped, *2*: 450
Underfeeding, *2*: 454
Undernutrition, *2*: 451
Uniformity, *2*: 1053
Unit, *2*: 1053
Unit operations, *1*: 13, 20–24
United States Department of Agriculture, (USDA), *2*: 986, 1054
  standards for grades of pickles, *2*: 1046
Unmolding, *2*: 477
Unsaponifiable, *2*: 463
  matter, *2*: 472
Upper limit of safety (UL), *2*: 202
Uric acid, *2*: 467
USDA, *2*: 950, 1016, 1019
USDA agricultural handbook, *2*: 476
USDA colors, *2*: 1024

4-Vinylguaiacol, *1*: 857
P-Vinylguaiaco, *1*: 861
Vacuum, *2*: 479
Vacuum steam-stripping, *1*: 154
Vacuum-packaging, *2*: 261–262
Valencene, *1*: 852–854
Valencia orange juice, *1*: 861
Valencia oranges, *1*: 853
Valeric acid, *1*: 182
Valine, *1*: 174
Vancomycin-resistant enterococci, *2*: 94
Vanilla, *2*: 404, 406, 409, 618–619
Vanillin, *1*: 197, 513
Vaporization, *1*: 162
Vapor-liquid equilibrium, *1*: 160
Variants, *2*: 470
Vegetable, *2*: 56, 59, 62, 406
Vegetable oil mills except corn, cottonseed, and soybean, *1*: 107
Vegetable oils, *1*: 997, 1001, 1003
Vegetables, mixed frozen, *2*: 994–995
  kinds, *2*: 994
  proportions, *2*: 995
Vegetarians, *2*: 202, 212
Venus mercenaria, *2*: 898
Veterinary, *2*: 456
Vicinal diketones, *1*: 182, 477–479
  biosynthesis, *1*: 471
Viili, *2*: 412, 424–425
Violations in confectionery products, *1*: 700
  choking hazards, *1*: 700
  filth contaminations, *1*: 700
  honey with chloramphenicol, *1*: 701
  undeclared ingredients, *1*: 700
Virgin olive oil, *1*: 753, 758
  carotenoid, *1*: 761–762
  chlorophyll, *1*: 765–766
  extraction process, *1*: 753
  phenols, *1*: 765–766
  quality, *1*: 763
  tocopherol, *1*: 761
  volatiles, *1*: 765
Virulence factors, *2*: 93–95
Viscosity, *2*: 374, 477, 637, 639, 641
Viscosity of chocolate, *1*: 683–684
Vital wheat gluten, *1*: 295
Vitamins, *1*: 921, 984, 993, 997–998, 1009, *2*: 365, 371, 388, 394, 458, 467
  A, *2*: 469
  B12, *2*: 470
  C, *2*: 469
  D, *2*: 469
  fat-soluble vitamins, *1*: 921
  folic acid, *2*: 470
  niacin, *2*: 469
  pantothenate, *2*: 469
  pyridoxine (B6), *2*: 470

riboflavin, *2*: 469
thiamin, *2*: 469
water-soluble vitamins, *1*: 921
Vitamin D, *2*: 202–205
from foods, both natural and fortified, *2*: 209, 212
from supplements, *2*: 202–204
skin biosynthesis, *2*: 209, 211–212
Vitamin D metabolites, *2*: 205
25-hydroxyvitamin D, *2*: 209
calcitriol (1,25-dihydroxyvitamin D), *2*: 205
VLDL, *2*: 383
VLE. *See* Vapor-liquid equilibrium
VO. *See* Volatile oil
Volatile compounds. *See* Flavor
Volatile oil, *1*: 148–149, 212
Volatiles, *1*: 828
Volume, *1*: 289, 317–318
Volvariella volvacea, *1*: 183

Washing, *1*: 582, 625–626
Water activity, *1*: 226, 232, 287, 291, 302
Water radiolysis, *1*: 126
Water supply, *1*: 230
Water, *1*: 425–428, 621, 625, *2*: 381, 457, 874
activity, *2*: 219, 254
diffusion, *2*: 237–238
holding capacity, *2*: 235, 245
ice, *2*: 595
loss, *2*: 235, 243, 252
purification treatments, *1*: 431
conventional lime treatment systems (CLTS), *1*: 427
ion exchange, *1*: 428
membrane technology, *1*: 427–428
quality, *1*: 427, 611
supplies, *1*: 425–426
Weed off-flavor, *1*: 745
Weight control, *2*: 180, 203, 207
Weight loss, *2*: 184–185, 207
Weight reduction, *2*: 106
Weight reduction products, *1*: 968
Wet corn milling, *1*: 100–101
Whale, *2*: 381
Wheat flour, *1*: 66
Wheat-based Asian noodle, *1*: 542–554
chemical properties comparison, *1*: 542
Wheat-salted Asian noodles, *1*: 543–545
flat noodles, *1*: 544
general manufacturing scheme, *1*: 545
thick noodles, *1*: 543
very thin noodles, *1*: 543
wide flat noodles, *1*: 544
Whey, *1*: 570–572, 574, 576, 578–584, 590, 596, 602, 608, 611, 622, 624–626, 630–631, *2*: 381, 467
Whey protein, *1*: 653–654, *2*: 465, 650, 652, 657, 660, 662, 696–698
denaturation, *2*: 653
Whisky (whiskey), *1*: 507
alchemist, *1*: 507
aqua vitae, *1*: 507
Scotch whisky, *1*: 513
uisge beatha, *1*: 507
usquebaugh, *1*: 508
water of life, *1*: 507
White, unripened cheeses, *1*: 637
WHO/FAO, *1*: 1021
Whole spices, *1*: 218
Wine, *1*: 182, 206, 918
Wines, brandy and brandy spirits, *1*: 109
Women's Health Initiative (WHI), *2*: 204
Woodchips, *1*: 490
Work-in-process, *1*: 234
World Health Organization (WHO), *1*: 11, 302, 1021
Wort, *1*: 443
acidification, *1*: 458
aeration, *1*: 447
aldehydes, *1*: 458
boiling, *1*: 447, 452, 455–456
carbohydrates, *1*: 467
clarification, *1*: 447, 457, 464
cooling, *1*: 447, 456, 459, 466
high-gravity, *1*: 468, 474
separation, *1*: 448–449, 452, 454
Wrappers, *2*: 869

Xanthine oxidase, *2*: 468
bovine, *2*: 468
caprine, *2*: 468
Xi mien, *1*: 543
X-ray detector, *1*: 216
Xylitol, *2*: 639

Yam noodles, *1*: 561
Yang-chun mien, *1*: 544
Yarrowia lipolytica, *1*: 197
Yeasts, *1*: 295–296, 356, *2*: 154, 267–270, 272–273, 287, 652, 656–659, 662
  alloploid, *1*: 470
  aneuploid, *1*: 470
  cell membrane, *1*: 468
  compressed, *Saccharomyces cerevisiae*, *1*: 361
  growth kinetics, *1*: 468
  immobilized, *1*: 186, 190
  lipid vesicles, *1*: 476
  plasma membrane, *1*: 470, 474, 484
  polyploid, *1*: 469–470
  saccharomyces boulardii, *2*: 154
  species in sourdough, *1*: 361, 363
Yellow-alkaline wheat-based Asian noodles, *1*: 545–546
Yersinia enterocolitica, *2*: 509
Yield, *1*: 570–571, 574–576, 579–580, 584, 619–621, 624, 628, *2*: 452, 456
Yi-mien, *1*: 545
Yinsi (silver threads) noodle, *1*: 543
Ymer, *2*: 416, 432
Yogurt, *1*: 12, 57, 59, *2*: 391, 412, 417, 432, 450, 474, 477, 495
  bacteriophage, *2*: 653, 659
  bio-yogurt, *2*: 412, 422
  common gums used, *1*: 60
  concentrated/strained yogurt, *2*: 432
  classification, *2*: 412
    Bulgarian buttermilk, *2*: 418
    concentrated/strained yogurt, *2*: 420–421
    drinking yogurt, *2*: 420
    set yogurt, *2*: 419
    stirred yogurt, *2*: 419
  consumption, U.S. per capita, *2*: 648
  culture, *2*: 653
    characteristics, *2*: 667
    Lactobacillus bulgaricus, *2*: 649, 652, 658–659, 665, 667
    live and active seal, *2*: 649, 668
    probiotic, *2*: 648, 669
    rods to cocci ratio, *2*: 652, 666
    ropy, *2*: 655, 660, 666
    Streptococcus thermophilus, *2*: 649, 652, 658–659, 665, 667
    survival, *2*: 667
  cup set, *2*: 648, 650, 665
  definition of, *2*: 648
  drinks, *2*: 649, 661
  drinking yogurt, *2*: 432
  effect of strength and weakness of milk components used in production, *2*: 435
  fermentation, *2*: 654–655, 664
    cup set, *2*: 648, 650, 656
    endpoint, *2*: 656, 660
    post-fermentation acidification, *2*: 664
    slow, *2*: 652–653, 659
    stirred style, *2*: 650, 656
    temperature, *2*: 655–656, 660, 665
  flavors used, *1*: 60
  frozen, *2*: 600
  frozen yogurt processing, *2*: 443
  gel strength
    ingredient effect, *2*: 652, 664
    processing effect, *2*: 655, 660, 662
  ingredients
    fruit preparation, *2*: 654, 658
    incorporation of, *2*: 654
    milk, *2*: 649
    stabilizer, *2*: 653, 661
    sweetener, *2*: 653–654
  long-life drinking processing, *2*: 442–443
  natural yogurt, *2*: 436
  nutritive value, *2*: 666
  organoleptic defects, *2*: 657–658
  packaging and storage, *2*: 659
  permitted colorings, *1*: 61
  petit Suisse/fromages frais, *2*: 434
  probiotic yogurt, *2*: 412, 434
  processing
    heat treatment, *2*: 652, 660, 662
    homogenization, *2*: 654
    shear, *2*: 660
  quality control, *2*: 659
  set-yogurt, *2*: 432
  set yogurt processing, *2*: 441–442
  shelf life, *2*: 649, 658, 664, 668
  standards, *1*: 61
  stirred yogurt processing, *2*: 441–442
  sweetened and fruit flavored yogurt, *2*: 412
  texture
    casein micelle, *2*: 662, 663

grainy, *2*: 654
ropy, *2*: 655, 660, 664
weak gel, *2*: 655, 660
yogurt mousse (aerated yogurt), *2*: 434
(Z)-1,5-octadiene-3-one, *2*: 732
(Z)-2-nonenal, *2*: 732
(Z)-4-heptenal, 2-acetyl-1-pyrroline, *2*: 728
Zinc, *2*: 473, 993, 1009